Defective Synthesis of Triglyceride Fatty Acids from 1-C-14 Acetate in the Well-controlled Stable Adult Diabetic

Allen R. Hennes, M.D., and Tommy W. Redding, B.S., Oklahoma City

In 1944, Stetten and Boxer demonstrated that synthesis of fatty acids from glucose in alloxan diabetic rats was approximately 5 per cent of normal. They further found that the quantity of glucose recovered in the urine was approximately equivalent to the amount which should have been utilized for synthesis of fatty acids. They concluded that the diabetic animal's failure to utilize glucose in the synthesis of fatty acids constituted a major metabolic defect in this disease.[1] In 1950, Brady and Gurin found that conversion of acetate to fatty acids was markedly impaired in the liver of alloxan diabetic rats, although synthesis of cholesterol was approximately normal.[2] Defective synthesis of fatty acids by alloxan diabetic or pancreatectomized animals has been amply confirmed in numerous in vivo and in vitro studies.[3,4] This defect has been easily corrected by pretreatment of the animals with insulin, at least when treatment has been prolonged for approximately forty-eight hours.[3,5]

Data on cholesterol synthesis have been less consistent, however, with some investigators reporting increased synthesis[6] and others normal synthesis in the diabetic animal.[2]

The present studies were undertaken to determine if abnormalities in lipid synthesis could be detected in the well-controlled stable adult diabetic as compared to subjects of a similar age without diabetes.

METHODS

All control subjects had a normal three-hour glucose tolerance test. All subjects were hospitalized and received a diet consisting of 250 gm. carbohydrate, 80 gm. protein and a total of 2000 to 2400 calories, depending on their weight. The weight of each subject was stable or almost so during the period of hospitalization. Diabetes was controlled in all cases with NPH insulin. The last dose of NPH insulin was given the morning prior to the start of the study. Fasting blood sugar was within the normal range in all but one diabetic subject. Clinical data on diabetic and control subjects are presented in tables 1 and 2. All food was withheld for the first eight hours of the experiment so that endogenous lipid metabolism could be determined. In all studies 100 μc. 1-C-14 acetate with 1 mM carrier sodium acetate was administered intravenously in a single rapid injection. Venous blood samples were obtained at one-half to two-hour intervals after the injection. Plasma lipids were extracted by the method of Folch et al.[7] The lipid samples were placed on silicic acid columns and were separated into four major lipid classes by the method of Barron and Hanahan.[8] In order of their elution from the column, these fractions were cholesterol ester, triglyceride fatty acid, free cholesterol, and phospholipid. Cholesterol was determined by the method of Carr and Drekter.[9] Triglyceride fatty acids were determined by the method of Van Handel and Zilversmit.[10] Each fraction from the column was saponified. After saponification, the nonsaponifiable material was extracted. The triglyceride fatty acid fraction was then acidified and the triglyceride fatty acids extracted separately.

RESULTS

Peak specific activity in plasma triglyceride fatty acids was reached in both nondiabetic and diabetic subjects between one and four hours after the start of the study except in one diabetic, in whom peak activity was not reached until eight hours after the start of the study. Despite a normal fasting blood sugar at the start of the experiment no diabetic incorporated a normal amount of C-14 into plasma triglyceride fatty acids. Peak specific activity in the five subjects without diabetes varied between 80 and 365 DPM/mg. of triglyceride fatty acids

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From the Medical Department, Veterans Administration Hospital, and University of Oklahoma School of Medicine, Oklahoma City, Oklahoma.

TABLE 1
Clinical data on diabetic subjects

	FBS	Age	Wt. (lbs.)	Duration of diabetes	Insulin dose	Complications
W.S.	82*	52	190	8 months	35 NPH	0
J.S.	127*	60	104	1 year	32 NPH	0
H.K.	109†	47	181	8-10 years	20 NPH	0
P.R.	109†	56	133	4 years	25 NPH	Mild peripheral neuropathy
W.M.	116†	56	192	5 years	20 NPH	0

*Normal 60-90 mg. per 100 ml.
†Normal 80-120 mg. per 100 ml.

TABLE 2
Clinical data on control subjects

	Age	Wt.	Diagnosis
J.B.	62	170	Addison's disease on maintenance therapy.
N.B.	65	169	Addison's disease on maintenance therapy.
J.E.	46	165	Convalescing from viral pneumonitis.
R.McC.	54	118	Hemorrhoids
L.S.	49	157	Treated pernicious anemia

with a mean of 225. Peak specific activity in the five diabetics varied between 3 and 50 DPM/mg. of triglyceride fatty acid with a mean of 21 (figure 1). Maximum per cent incorporation of C-14 into plasma triglyceride fatty acids, which takes into account plasma volume and level of triglyceride fatty acids as well as specific activity, was similarly markedly depressed in the diabetic subjects as compared to subjects without diabetes (figure 2). Incorporation of C-14 into plasma triglyceride fatty acids was in fact six times as great in subjects without diabetes as in the diabetic subjects. All triglyceride fatty acid data on diabetic and control subjects are presented in tables 3 and 4.

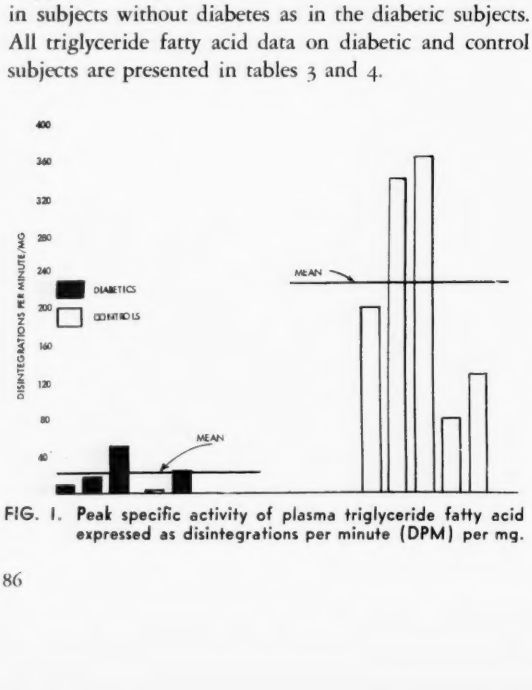

FIG. 1. Peak specific activity of plasma triglyceride fatty acid expressed as disintegrations per minute (DPM) per mg.

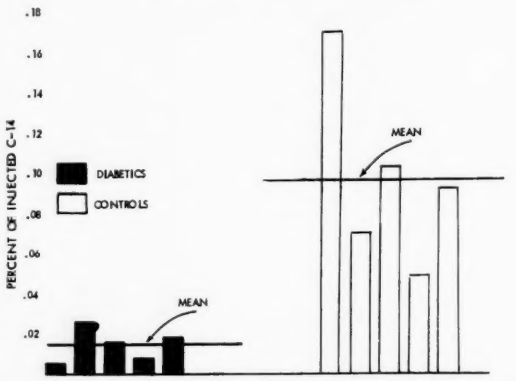

FIG. 2. Per cent of injected C-14 incorporated into plasma triglyceride fatty acids.

The lowest incorporation of C-14 into triglyceride fatty acids was seen in the best-controlled diabetic subject in whom the fasting blood sugar had been consistently between 70 and 80 mg. per 100 ml. for at least ten days prior to the study. In this subject incorporation of C-14 into fatty acids of ester cholesterol and phospholipid was also determined. Almost no activity was found in these fatty acids.

No diabetic subject showed increased synthesis of cholesterol. In fact, in two of five, the peak specific activity of free cholesterol was less than that reached by our lowest nondiabetic subject. Peak specific activities of three of the five diabetic subjects and of the normal subjects were within the normal range found by Gould et al.[11] with 1-C-14 acetate. Peak specific activities in diabetic subjects varied between 23 and 174 DPM/mg. free cholesterol. Peak specific activity in subjects without diabetes varied between 90 and 522 DPM/mg. free cholesterol (figure 3). Total incorporation of C-14 into plasma-free cholesterol also showed considerable overlap between the group with diabetes and that without diabetes (figure 4). All free cholesterol data in diabetic and nondiabetic subjects are presented in tables 5 and 6.

Specific activity in ester cholesterol lagged behind that in free cholesterol and was still increasing in most studies at the end of the twenty-four-hour period. In one diabetic subject, the specific activity at twenty-four hours was lower than at eight hours. The usual precursor: product relationship between free and esterified cholesterol was seen in all subjects.

One subject (W.S.) was studied again after twelve days of a 400 gm. carbohydrate, 80 gm. protein, 2200 calorie diet. He was also given 7 units Regular Insulin and 50 gm. additional carbohydrate ten hours before the start of the study. In spite of this marked increase

TABLE 3
Triglyceride fatty acid data in diabetic subjects

	Hours																							
	½			1			2			3			4			6			8			24		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
W.S.				93	2	.003	78	3	.004	75	3	.004	109	3	.005	95	2	.003	99	0	0	57	0	0
J.S.				103	21	.019	90	24	.019	114	14	.014	91	23	.018									
H.K.	36	9	.005				65	14	.014				49	17	.013	92	11	.016	34	27	.014	46	13	.009
P.R.	82	2	.002	78	6	.005	84	9	.008				45	8	.004	41	3	.001						
W.M.	55	8	.007	35	20	.011	45	50	.036				38	35	.021	77	11	.014				25	29	.012

a = mg. per 100 ml. of triglyceride fatty acids
b = DPM/mg. triglyceride fatty acids
c = per cent of injected C-14 incorporated in plasma triglyceride fatty acids

TABLE 4
Triglyceride fatty acid data in control subjects

	Hours																							
	1			2			3			4			6			8			24					
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
J.B.	50	201	.144	61	130	.109				78	15	.016	43	14	.009	61	17	.015						
J.E.	96	80	.106	79	62	.068	57	15	.012	90	42	.053	85	8	.009	130	3	.005	87	2	.002			
N.B.	28	81	.032	73	22	.023				15	342	.073	173	10	.025	63	8	.007						
R.McC.	81	16	.013	14	365	.056	84	12	.01	9	40	.004	60	63	.037									
L.S.	9	135	.016	93	78	.096	94	50	.062	87	38	.044												

a = mg. per 100 ml. of triglyceride fatty acids
b = DPM/mg. triglyceride fatty acids
c = per cent of injected C-14 incorporated in plasma triglyceride fatty acids

TABLE 5
Free cholesterol specific activities in diabetic subjects (DPM/mg. cholesterol)

	Hours							
	½	1	2	3	4	6	8	24
P.R.	4	17	12		18	16	23	14
H.K.	13	124	100		55	76	53	16
W.M.	6	7	35		32	42	40	28
W.S.		102	140	156	178	128	115	71
J.S.		101	174	152	155			

TABLE 6
Free cholesterol specific activities in control subjects (DPM/mg. cholesterol)

	Hours						
	1	2	3	4	6	8	24
J.B.	84	97		121	79	44	
N.B.	276	404		522	514	403	
J.E.	105	184	176	174	148	125	85
R.McC.	46	78	90	49	54		

in carbohydrate, insulin requirement remained essentially the same (35-37 units NPH daily), as prior to the start of the diet. The 400 gm. carbohydrate diet appeared to increase slightly the incorporation of C-14 into triglyceride fatty acids as well as into free and esterified cholesterol (table 7). The increase in peak specific activity of plasma triglyceride fatty acids was, however, primarily a reflection of a striking decrease in levels of plasma triglyceride fatty acids associated with the very low fat intake. This resulted in less dilution of newly synthesized radioactive triglyceride fatty acids with unlabeled lipid and a marked increase in specific activity. The percentage of injected C-14 incorporated into plasma triglyceride fatty acids increased only from .005 per cent to .011 per cent, a value approximately one fifth of that reached by our lowest control subject. Therefore, the actual increase in synthesis of triglyceride fatty acids was very slight. That adequate insulin activity was present, at least in this experiment, is shown by a definite fall in blood sugar from 72 to 52 mg. per 100 ml. during the first four hours of the study.

DISCUSSION

These studies demonstrate that synthesis of cholesterol is normal or even less than normal in the stable adult diabetic with normal fasting blood sugar. Therefore, the vascular complications which occur in diabetics in spite of good control cannot be attributed primarily to increased synthesis of cholesterol. If abnormalities in cholesterol metabolism are of importance in the genesis of these complications, processes of removal and degradation may be of more importance than those of synthesis. It is perhaps of significance, in this regard, that Wong and Van Bruggen[12] have recently shown that degradation of cholesterol is abnormally slow in the alloxan diabetic rat.

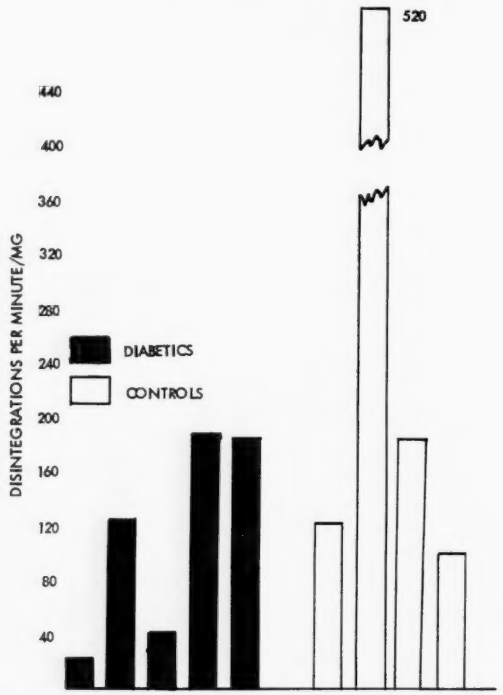

FIG. 3. Peak specific activity of plasma-free cholesterol.

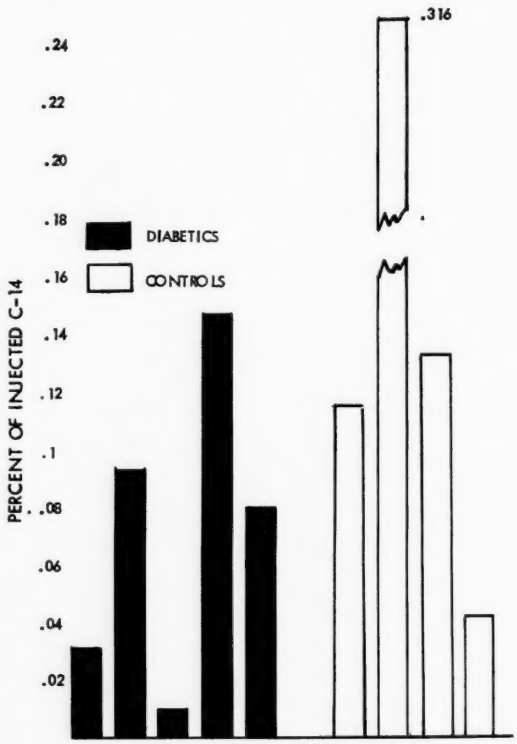

FIG. 4. Per cent of injected C-14 incorporated into plasma-free cholesterol.

The synthesis of triglyceride fatty acids from C-14 acetate in the diabetic is uniformly much less than that of our control group. It is striking that this defect occurs in spite of a normal fasting blood sugar and in spite of apparently unimpaired oxidation of glucose as demonstrated in studies with C-14 glucose in diabetics of this type.[13,14] Very low levels of activity in ester cholesterol and phospholipid fatty acids in the one diabetic in whom these determinations were made suggest that the defect is actually in synthesis of fatty acids rather than only in synthesis of triglyceride fatty acids. In previous studies, Shreeve, Hennes and Schwartz[15] have shown that oxidation of acetate is perfectly normal in well-controlled diabetics. It is unlikely that the defect in fatty acid synthesis is due to an enlarged acetate pool or to a generalized defect in activation of acetate.

TABLE 7

Effect of increased carbohydrate (? decreased fat) intake on synthesis of lipids from 1-C-14 acetate in a diabetic subject

	Diet	
	250 gm. carbohydrate	400 gm. carbohydrate
Peak specific activity (DPM/mg.)		
Triglyceride fatty acids	3	67
Cholesterol	178	211
Per cent incorporated into plasma lipids		
Triglyceride fatty acids	.005	.011
Cholesterol	.147	.165

It is interesting that Bierman, Dole and Roberts[16] have also shown that control of fasting blood sugar does not necessarily control abnormalities in lipid metabolism, since they were able to demonstrate elevated nonesterified fatty acid levels in controlled diabetics.

Thus a normal fasting blood sugar does not mean that lipid metabolism is also normal. Nor are levels of plasma lipids an adequate indication of the underlying state of lipid metabolism since, in all instances, levels of cholesterol and triglyceride fatty acids were normal in these diabetics. It appears that when synthesis of lipids is slow, degradation is also slow so that normal plasma levels of lipids usually result. It may be that *turnover* of lipids is a more accurate reflection of the underlying state of metabolism in the diabetic subject and turnover at least of triglyceride fatty acids and at times of cholesterol appears to be abnormally slow.

There are several possible reasons for the defective synthesis of plasma lipids seen in these diabetic subjects. It is

possible that larger doses of insulin or increased amounts of carbohydrate may be required to correct this defect, although our one attempt with a regimen of this type was less than successful. Perhaps insulin must go directly to the liver rather than to the periphery. However, parenteral injections of insulin correct the defective lipid synthesis in the animal with experimental diabetes. We must also consider the possibility that the defect in lipogenesis in the stable adult diabetic cannot be corrected completely by insulin, either because of an enzymatic defect or because another hormone is also required. The diabetes of the stable adult diabetic might then be secondary to defective utilization of glucose in the synthesis of fat. This possibility, first suggested by Lawrence,[17] is supported by the data presented in this study.

SUMMARY

The synthesis of plasma lipids from 1-C-14 acetate has been studied in nondiabetic subjects and in diabetics controlled with long-acting insulin. Lipids were fractionated on silicic acid columns.

1. No diabetic was found to synthesize more than normal amounts of plasma-free cholesterol from 1-C-14 acetate. In fact, two of the five diabetics synthesized less than normal amounts of plasma-free cholesterol.

2. All diabetics showed a striking decrease in synthesis of plasma triglyceride fatty acids.

3. These abnormalities occur despite a normal fasting blood sugar.

4. The presence of a normal fasting blood sugar and normal plasma lipid levels does not indicate that the lipid metabolism of the diabetic is normal.

SUMMARIO IN INTERLINGUA

Defectos del Synthese de Acidos Grasse Triglyceridic ex Acetato a 1-C-14 in le Ben-Regulate Stabile Diabetico Adulte

Esseva studiate le synthese de lipidos plasmatic ab acetato a 1-C-14 in subjectos non-diabetic e in diabeticos stabilisate per medio de insulina a action prolongate. Le lipidos esseva frationate in columnas a acido silicic.

1. Esseva trovate que nulle del diabeticos synthetisava plus que normal quantitates de cholesterol libere del plasma ex acetato a 1-C-14. De facto, duo del cinque diabeticos synthetisava minus que normal quantitates de cholesterol libere del plasma.

2. Omne le diabeticos manifestava un frappante reduction del synthese de acidos grasse triglyceridic del plasma.

3. Iste anormalitates occurreva in despecto de normal valores del sucro sanguinee in stato jejun.

4. Le presentia de valores normal del sucro sanguinee e de normal nivellos del lipidos del plasma non significa que le metabolismo lipidic del diabetico es normal.

ACKNOWLEDGMENT

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Histopathologic Lesions Associated with Sulfonylurea Administration

J. M. B. Bloodworth, Jr., M.D., and George J. Hamwi, M.D., Columbus, Ohio

Despite the widespread use of the sulfonylurea drugs very few complete postmortem examinations of patients receiving these drugs have been reported.[1-4] Therefore, when a sufficient number of autopsy protocols became available, a study of the clinical, laboratory and histologic changes associated with sulfonylurea administration was undertaken. A specific search was made in each patient for all previously reported toxic effects of the sulfonylureas.[1,5,6] Because of the chemical similarity between the sulfonylureas and the sulfonamides, a careful search was also made for lesions which have been attributed to sulfonamide therapy.[7-11] This is a report of the first twenty sulfonylurea-treated patients on postmortem examination at The Ohio State University.

MATERIALS AND METHODS

Twenty consecutive autopsied patients who received sulfonylurea therapy for diabetes mellitus were studied. Patients were treated by members of the Department of Medicine of The Ohio State University and, with the exception of cases 7 and 10, were treated at the University Hospital and/or University Clinics. One patient (case 10) received carbutamide; all others were treated with tolbutamide. Extensive clinical histories, including many special laboratory studies, were available for study.

Postmortem examinations were done at the University Hospital under the direct supervision of the senior author. With the exception of case 7, in which permission for examination of the head was not granted, all patients underwent complete postmortem examination. Tissues were formalin-fixed and paraffin sections were stained with hematoxylin and eosin. Additional tissue blocks from all diabetic patients autopsied at the University Hospital are routinely preserved in an archive collection, and use was made of this material when indicated. Multiple tissue blocks from the heart, liver, pancreas and kidney of each case were examined histologically. Serial sections of selected areas of the heart were made to differentiate myocarditis from acute myocardial infarction when both were present in the same heart. Special studies of the pancreatic islets will be reported elsewhere.

The case reports are presented in table 1. Patients are arranged according to duration of therapy with the exception of cases 18, 19, and 20. These patients had received large quantities of tolbutamide, but therapy had been discontinued two months prior to death in case 18, fifteen and one-half months prior to death in case 19 and three months prior to death in case 20.

Diabetes control was considered good (G) when 1-2 gm./day of the sulfonylurea drug were sufficient to produce essentially glucose-free urine, fair (F) if there was occasional 1-3+ glycosuria and poor (P) when hyperglycemia and excessive glycosuria necessitated institution of insulin therapy.

The second part of table 1 shows the distribution of granulomatous lesions, graded 1-4+ according to frequency and/or severity.

The third part of table 1 shows the incidence of other frequently encountered pathologic conditions. No attempt was made to grade the degree of involvement by these conditions.

All clinical or laboratory manifestations of disease found in the sulfonylurea-treated patients which resembled the previously reported untoward effects of either the sulfonylureas or the sulfonamides are included in the case reports or the discussion.

Fifty consecutive adult diabetics who had never received any of the oral hypoglycemic drugs, and who underwent postmortem examination during the same period of time as the sulfonylurea-treated patients, were used as controls. These control patients were subjected to study in a manner identical to that used for the sulfonylurea-treated patients. The autopsies of the sulfonylurea-treated diabetics and the diabetic controls were performed by the same physicians. Histopathologic ex-

Presented at the Nineteenth Annual Meeting of the American Diabetes Association in Atlantic City on June 7, 1959.

From the Division of Pathologic Anatomy of the Department of Pathology and the Division of Endocrinology of the Department of Medicine, The Ohio State University, Columbus, Ohio.

TABLE 1

Case reports, sulfonylurea-treated patients

Case number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Part one																				
Age	67	59	71	73	65	50	47	55	65	62	78	62	76	67	53	48	57	56	48	64
Color, (W = white, N = Negro)	N	W	W	W	W	N	W	N	N	W	W	W	W	W	W	W	N	N	N	W
Sex	F	F	F	F	M	M	M	F	M	F	M	M	F	M	F	M	M	F	F	M
Insulin dosage prior to sulfonylurea therapy, units	10	10	0	15	20	10	0	15	40	20	35	10	0	0	30	25	0	0	20	0
Duration of sulfonylurea therapy, days	456	395	365	365	350	305	182	144	60	55	33	26	15	11	11	6	4	340	198	300
Total quantity of sulfonylurea ingested, grams	459	385	365	460	864	305	225	144	120	55	72	29	63	54	33	18	8	200	395	305
Diabetes control by sulfonylurea drug	G	G	G	G	G	G	G	G	G	G	P	G	F	F	P	P	F	G	G	G
Part two																				
Myocarditis	3+		2+	+	+	+		3+		+		2+		2+	2+	±	2+			±
Blocks with myocarditis / Blocks examined	6/17	0/5	4/11	1/11	2/14	2/9	0/6	3/3	0/8	2/6	0/7	3/7	0/22	4/7	4/7	1/15	3/6	0/14	0/4	1/5
Microgranulomata, heart	4+		+					+		+		+	+	±		+	+			
Microgranulomata, liver	4+		3+		3+	3+	±	4+	+	2+	+	3+	3+	+	4+	2+	3+	+	+	+
Gastrointestinal granulomata	3+		2+					+					+	+	3+	+		2+		
Trabeculitis of the spleen					+		+				+	+	+	+		2+				
Microgranulomata, adrenal	3+		+	+				+		+			+	+	3+					
Microgranulomata, kidney	3+		+	+	+	3+				+	+	+	+		3+	2+	3+	±		+
Granulomatous interstitial nephritis				+	+	+														
Part three																				
Hypertension	+	+	+	+	+	+				+			+	+	+	+	+			+
Myocardial infarction (old) / (recent)		+ / +	+	+	+					+		+				+				+
Congestive heart failure	+	+		+	+	+						+	+		+	+		+		
Pneumonia						+		+	+	+	+				+		+	+	+	
Pulmonary thromboembolism				+					+				+	+		+	+			
Pleural effusion	+	+	+		+		+					+			+	+		+		+
Pulmonary edema	+		+						+			+	+							
Per cent hyalinization of islets of Langerhans		5	50		75					40				5						
Adrenal cortical hyperplasia		+	+	+	+			+	+		+	+	+	+	+	+			+	+
Diabetic glomerulosclerosis	+	+	+		+	+	+			+		+	+	+	+	+		+	+	
Postmortem blood urea nitrogen (mg./100 ml.)	19	62	15	62	25	98	69	6	96	25	149	65	—	19	63	130	30	47	118	31

amination of the tissues of both groups was made by the senior author.

RESULTS AND DISCUSSION

Histopathologic examination of the tissues from our sulfonylurea-treated patients revealed a high incidence of "granulomatous" lesions.

Microgranulomata were the most frequent granulomatous lesions found. These lesions occurred most often in the liver and kidney but also quite frequently in the heart, adrenal and gastrointestinal tract. The microgranulomata were composed of well-circumscribed collections of 20-100 cells interspersed in a sparse, granular eosinophilic debris. The predominant cell was the acidophilic histiocyte with lesser numbers of lymphocytes, plasma cells, neutrophils and often eosinophils. The lesions

were usually perivascular.

In addition, diffuse infiltrates of the same types of cells comprising the microgranulomata were seen in the heart (myocarditis) and the kidney, (interstitial nephritis). Occasionally, diffuse granulomatous infiltrates were present in other organs.

The microgranulomata and diffuse granulomatous infiltrations were identical in nature and location with those previously reported in sulfonamide-treated patients,[7–10] although only one patient in the group (case 2) was known to have received sulfonamides. However, the sulfonylurea-associated granulomatous involvement appeared somewhat less extensive than in the reports of sulfonamide-induced lesions. The more severe changes of sulfonamide hypersensitivity such as polyarteritis were not seen.[11]

The incidence of granulomatous lesions in sulfonylurea-treated patients and diabetic controls is reported in table 2. The sulfonylurea-treated cases have been divided into two groups: "long-term therapy" (twelve to fifteen months, cases 1 through 9); and "short-term therapy" (four to fifty-five days, cases 10 through 17). Cases 1 through 17 comprise the "total series."

Granulomatous lesions occurred more often after sulfonylurea therapy than expected in the general autopsy population, or in the diabetic control patients who did not receive the sulfonylurea drugs. In addition, granulomatous lesions found in the control cases were rarely as extensive as those in sulfonylurea-treated cases.

TABLE 2

Comparison of sulfonylurea treated patients with diabetic controls. Granulomatous lesions

	Sulfonylurea therapy			
	Long-term over 100 grams	Short-term less than 100 grams	Total series	Diabetic controls
Number of patients	9	8	17	50
Average sulfonylurea administration, grams	370	42	215	None
	Per cent	Per cent	Per cent	Per cent
Myocarditis	67	63	65	34
Microgranulomata, heart	33	63	47	18
Microgranulomata, liver	67	100	82	22
Gastrointestinal granulomata	33	50	41	30
Trabeculitis of spleen	22	63	41	20
Microganulomata, adrenal	44	50	47	48
Microganulomata, kidney	56	88	71	30
Granulomatous interstitial nephritis	33	0	18	6

It is apparent that neither duration nor quantity of sulfonylurea administration alone is related to the incidence or severity of the granulomatous lesions. For instance, the tissues in case 2 were free of lesions after thirteen months of therapy. However, heart, liver and kidney granulomata were more frequent in the short-term group.

The pathogenesis of the granulomatous lesions remains uncertain although similar lesions following sulfonamide administration have been attributed to hypersensitivity.[12–16]

The incidence of other frequently encountered pathologic conditions found in the sulfonylurea-treated patients and the diabetic controls is given in table 3. The significance of these conditions, as well as the local characteristics of the granulomatous lesions and previously reported untoward effects of the sulfonylurea drugs, will be discussed by organ systems.

Cardiovascular system: Microgranulomata were found in the subepicardial, the subendocardial and the septal

TABLE 3

Comparison of sulfonylurea-treated patients with diabetic controls. Incidence of other commonly encountered pathologic conditions

	Sulfonylurea therapy			
	Long-term over 100 grams	Short-term less than 100 grams	Total series	Diabetic controls
Number of patients	9	8	17	50
Average age, years	61	63	62	61
Sex, per cent male	44	63	53	52
	Per cent	Per cent	Per cent	Per cent
Hypertension	67	75	71	64
Myocardial infarction	56	50	53	60
Congestive heart failure	56	50	53	58
Pneumonia	33	50	41	42
Pulmonary thromboembolism	22	50	35	16
Pleural effusion	56	38	47	42
Pulmonary edema	33	25	29	30
Ascites	44	25	35	34
Peptic ulcer	11	25	18	8
Significant chronic pancreatitis with atrophy	22	25	24	14
Significant hyalinization of pancreatic islets	22	13	18	16
Adrenal cortical hyperplasia	67	75	71	56
Diabetic glomerulosclerosis	67	75	71	62
Blood urea nitrogen, above 100 mg./100 ml.	0	25	12	30
Cerebral vascular accident	13	50	29	36

tissues of the heart. They were present in 47 per cent of the sulfonylurea-treated patients comprising this report, but in only 18 per cent of the diabetic controls. In several cases there was necrosis of individual myocardial fibers, or part of a fiber, with a surrounding cellular infiltrate identical with that of the microgranuloma. These necrotic lesions were considered a variant of the microgranuloma (figures 1, 2, 3).

In addition to the well-circumscribed microgranulomata found in the heart, eleven of the sulfonylurea-treated patients had a diffuse infiltration of the myocardium by acidophilic histiocytes, lymphocytes and neutrophils, with lesser numbers of plasma cells and eosinophils. Some hearts contained multiple foci of myocarditis. The larger areas of myocarditis were several millimeters in diameter, while the small areas consisted of infiltrates of twenty to fifty cells between the intact myocardial fibers. Because of the similar type of cellular infiltration and the common occurrence of both microgranulomata and myocarditis in the same heart, it is tempting to postulate that these lesions represent the same pathologic process. However, because of the potentially fatal nature of myocarditis and the uncertainty of the etiology, the diffuse cellular infiltrates of the myocardium have been listed separately. In addition, as shown by Fawcett[16] in his work on sulfonamides, one must exercise great care in attributing myocarditis to any drug.

Myocarditis was present in 65 per cent of the sulfonylurea-treated patients, but in only 34 per cent of the diabetic controls—a significant difference. In two patients, cases 1 and 3, the myocarditis was at least contributory to death. However, in most cases the myocar-

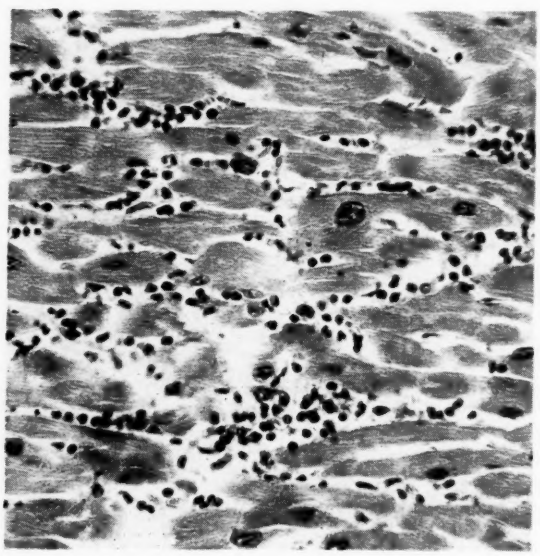

FIG. 1. Myocarditis, case 1. Hematoxylin and eosin X 260.

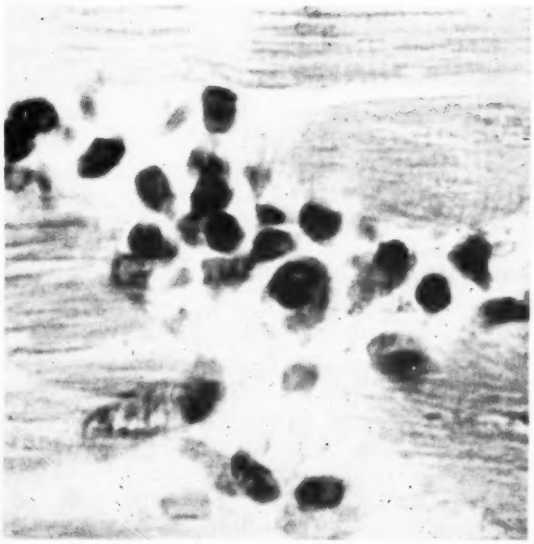

FIG. 2. Myocarditis, case 1. A higher magnification of figure 1 shows acidophilic histiocytes, lymphocytes, and plasma cells typical of the cellular infiltrates. Hematoxylin and eosin X 1,330.

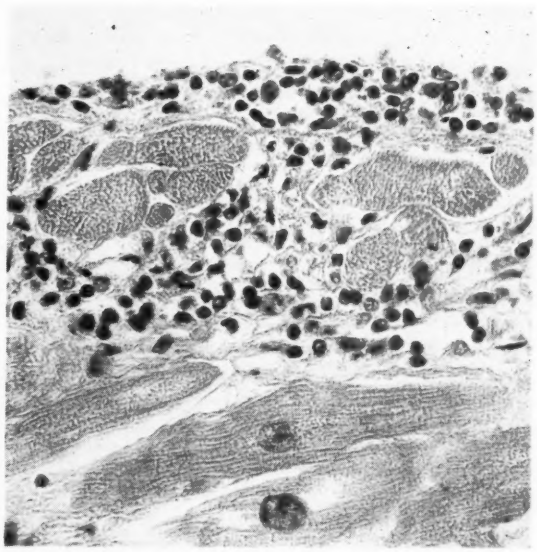

FIG. 3. Subendocardial microgranuloma of the heart, case 3. The cellular infiltrate is composed of acidophilic histiocytes, lymphocytes, and plasma cells. The myocardial fibers remain intact. Hematoxylin and eosin X 430.

ditis appeared to be an incidental finding since other more obvious causes of death were apparent. Many additional cases must be studied before a definite relationship between myocarditis and the sulfonylureas can be established. Nonetheless, these patients, as well as the previously reported cases of myocarditis occurring in patients receiving carbutamide,[4,17] emphasize the importance of careful examination of the heart.

Pericarditis was present in four cases (5, 7, 17, and 20); in two cases the pericarditis was secondary to myocardial infarction. Mural thrombi secondary to myocardial infarction were found in the hearts of cases 2, 4, 9, 14, and 20. Hypertension, myocardial infarction, congestive heart failure, pericarditis and mural thrombi seen in the sulfonylurea-treated patients comprising this report were in keeping with the expected incidence of such conditions in diabetic patients of this type and age.

Epicardial petechiae[18] and myocardial hemorrhages[19] have been reported in sulfonylurea-treated dogs. Paroxysmal auricular tachycardia has been observed in a patient treated with the sulfonylureas.[20] None of these conditions was seen in the sulfonylurea-treated patients comprising this study.

Respiratory system: A single instance of peribronchial granuloma was observed as part of the diffuse granulomatous involvement of case 1. No other granulomatous lesions of the respiratory system were seen in the sulfonylurea-treated patients comprising this report.

There were six cases of thromboembolic disease in the sulfonylurea-treated patients of this series, an incidence of 35 per cent. This incidence is somewhat higher than usually reported in autopsy statistics and approximately double the incidence of thromboembolism, 16 per cent, found in the diabetic controls.

Pulmonary edema and pleural effusion[21] have been reported in animals treated with sulfonylureas. However, the incidence of these lesions in the present sulfonylurea-treated patients was almost identical with that in the diabetic controls. Other respiratory lesions observed in the sulfonylurea-treated patients were not unexpected in the type of patients examined.

Gastrointestinal tract: Six of the sulfonylurea-treated patients had microgranuloma or diffuse cellular infiltrates of a similar nature in the mucosa and submucosa of the small intestine and colon. In no instance was the infiltration associated with ulcerations or symptoms. Two carbutamide-treated patients with comparable granulomatous lesions in the "abdominal organs" have been previously reported.[4,17]

Nausea and vomiting, superficial ulcerations of the gastrointestinal mucosa and gastrointestinal bleeding have occurred in animals receiving sulfonylurea therapy.[19,23-34] Mild gastrointestinal symptoms, usually limited to anorexia, nausea and vomiting, have occurred occasionally in patients.[5,6] Ascites and gastrointestinal symptoms occurring in the sulfonylurea-treated patients here reported were all explained by specific causes such as heart failure or carcinoma and cannot be attributed to the sulfonylureas.

At least four patients have been reported in whom the initial development, or exacerbation, of a peptic ulcer with gastrointestinal bleeding occurred while the patient was receiving tolbutamide.[25-27] Three peptic ulcers were encountered in the sulfonylurea-treated patients comprising this report. The history of peptic ulcer antedated the initiation of the sulfonylurea therapy in two patients, and one of these ulcers (in case 7) healed while the patient was receiving sulfonylurea therapy. The third patient, case 12, had an episode of gastrointestinal bleeding while receiving tolbutamide and a duodenal ulcer was demonstrated by X ray. The ulcer apparently healed while the patient was still receiving tolbutamide, since it was not found at autopsy.

Other pathologic conditions of the gastrointestinal tract included mesenteric artery thrombosis, case 4, and infarction of the colon without a grossly demonstrable vascular lesion, case 7.

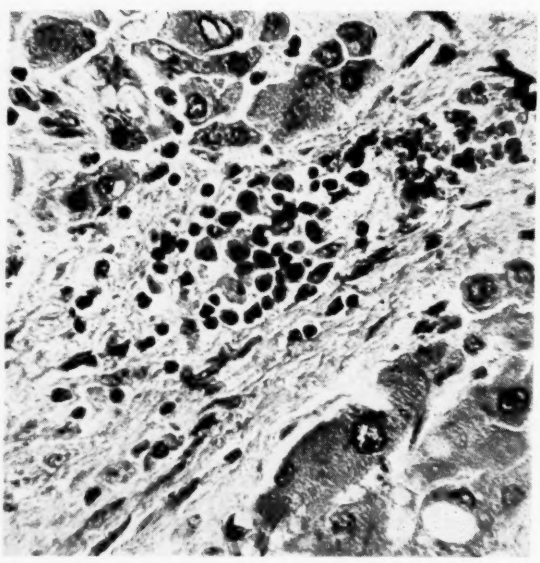

FIG. 4. Microgranuloma of the porta hepatica, case 11. The lesion is composed of a well-circumscribed collection of acidophilic histiocytes, lymphocytes and plasma cells located next to a small vessel at the end of a portal space. Hematoxylin and eosin X 440.

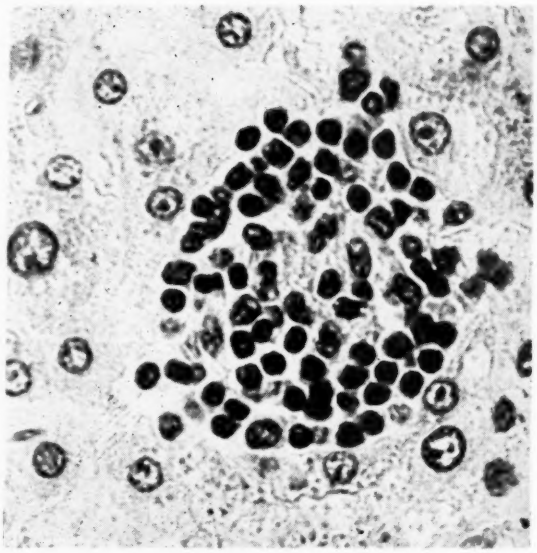

FIG. 5. Microgranuloma of the hepatic parenchyma, case 1. The lesion is composed of two to three disintegrating liver cells surrounded by a well-circumscribed collection of acidophilic histiocytes, lymphocytes, and plasma cells. Hematoxylin and eosin X 800.

Liver: Microgranulomata were found in 82 per cent of the sulfonylurea-treated patients, while similar lesions were found in only 22 per cent of the diabetic controls (figures 4, 5). Despite this high incidence of microgranulomata there were no related clinical symptoms or laboratory abnormalities. When the microgranulomata occurred in portal areas, they were differentiated from cholangitis by their well-circumscribed nature and the presence of both acidophilic histiocytes and eosinophils. The microgranulomata found among the liver cells of the lobule were usually composed of several necrotic liver cells surrounded by a well-circumscribed cellular infiltrate with a predominance of acidophilic histiocytes. In some cases, 1, 3, 6, 7, 12, 14, and 16, the portal areas were often infiltrated with eosinophils. There are but two prior reports of patients showing granulomatous lesions of the liver.[4,17] Both patients were receiving carbutamide.

Fatty infiltration of the liver, focal liver necrosis, jaundice, prolongation of the prothrombin time with hemorrhagic diathesis, fall in the serum albumin, and abnormalities of the liver function tests have been reported in animals, usually the dog, treated with the sulfonylurea drugs.[19,24,28–32]

Jaundice and abnormalities of liver function tests have also been reported in patients following administration of the sulfonylureas.[1,5,33] Positive histologic studies have been limited. Fatty infiltration was found in the liver tissue removed by needle biopsy from three patients receiving tolbutamide.[34] Another patient receiving carbutamide had focal necrosis and bile stasis in a biopsy specimen of liver and still another patient had diffuse hepatitis and bile stasis at autopsy.[20] Many negative reports dealing with liver structure and function after sulfonylurea therapy have also appeared.[1,6,35–37] Evaluation of the clinical history, laboratory data and autopsy findings failed to demonstrate previously reported liver lesions in the sulfonylurea-treated patients comprising this report.

A search was made for intracytoplasmic liver cell inclusions as reported by Sirek et al.[19] after tolbutamide administration to dogs; none was found.

In two patients of this series, cases 7 and 11, nutritional cirrhosis was present prior to initiation of sulfonylurea therapy. The sulfonylurea therapy did not appear to alter the course of the cirrhosis of either patient. However, one of these patients, case 11, developed hepatic insufficiency with jaundice in association with massive sepsis. This occurred two weeks after the discontinuation of the sulfonylurea therapy.

Pancreas: Two sulfonylurea-treated patients, cases 5 and 16, had typical microgranulomata in the pancreas. Acute purulent pancreatitis was present in cases 4 and 11. Three patients, cases 1 (figure 6), 15 and 16, had severe chronic pancreatitis with extensive fibrosis and atrophy. Three patients, cases 3, 5, and 10, had extensive hyalinization of the pancreatic islets. However, none of these lesions could be correlated with the clinical response of the patient to the sulfonylurea drugs.

Three patients (cases 11, 15, and 16) could not be controlled by tolbutamide while three patients (cases 13, 14, and 17) gave only a fair response. The pancreas of cases 15 and 16 showed extensive long-standing pancreatic destruction. However, the pancreas in case 1 showed a comparable degree of pancreatic destruction and yet the patient had a good clinical response to tolbutamide. In case 11 there was acute purulent pancreatitis at autopsy but no indication of pancreatic pathology of sufficient duration to have influenced the drug response at time of the failure of the therapeutic trial. Extensive hyalinization of the pancreatic islets did not appear to alter the therapeutic response to the sulfonylurea drug. All patients showing extensive hyalinization of the pancreatic islets (cases 3, 5, and 10) gave a good therapeutic response.

A detailed study of the effect of the sulfonylurea drugs on the pancreatic islet cytology was not made. Based on the limited material available, it appears that

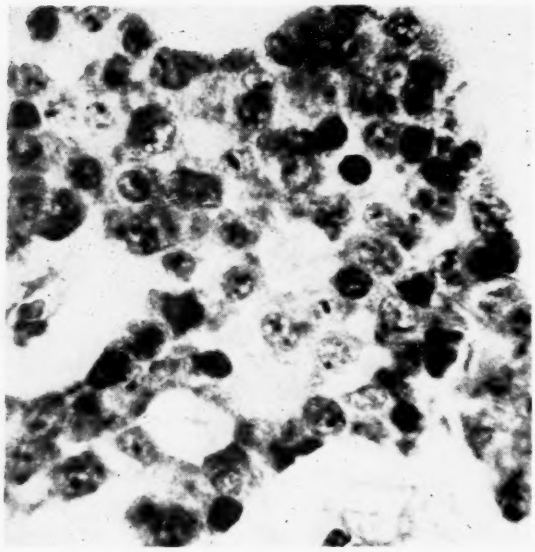

FIG. 6. Alpha cell hyperplasia of pancreatic islet, case 1. This islet is composed of approximately 85 per cent alpha cells which can be identified by the dark black alpha granules seen in most of the cells. These granules are red in the tissue section. Two of the rare beta cells, seen in the center of the photomicrograph, have abundant pale cytoplasm because the beta granules are only lightly stained. Rhodocyan stain X 900.

there is an increase in the percentage of islet alpha cells at the expense of the beta cells one to two weeks after instituting sulfonylurea therapy and a return to the normal proportion of alpha cells one to two weeks after discontinuation of sulfonylurea therapy.

Hematopoietic system: Seven of the sulfonylurea-treated patients comprising this report had "trabeculitis" of the spleen. This is an incidence of 41 per cent, while only 20 per cent of the diabetic controls showed trabeculitis. This lesion was first described by More et al.[9] in patients treated with the sulfonamide drugs. Trabeculitis consists of edema and fibrinoid necrosis of the trabecular collagen with fragmentation of elastic fibers and infiltration of acidophilic histiocytes, plasma cells, lymphocytes, neutrophils and eosinophils. This lesion is difficult to delineate and its significance, therefore, uncertain. Similar changes have not been reported previously in the spleens of animals or patients receiving sulfonylureas.

The administration of the sulfonylureas to animals has occasionally caused a fall in the hematocrit, hemoglobin and the circulating erythrocytes and leukocytes.[21] In patients treated with the sulfonylureas there have been occasional reports of agranulocytosis, leukopenia, acute anemia and thrombocytopenic purpura.[1,5,6,30,38–40] Among other patients there are instances of fatal aplastic anemia,[41] toxic depression of the bone marrow followed by the development of acute granulocytic leukemia,[42] and hemolytic anemia.[20]

Study of the reticuloendothelial system of the sulfonylurea-treated patients in this series showed diffuse reticulum cell hyperplasia in cases 1 and 9; and eosinophilia of the bone marrow in cases 4, 7, and 15. However, the clinical history, laboratory data and autopsy findings failed to provide evidence of frank hematopoietic disease. One patient, case 15, had an episode of hemolytic anemia beginning two weeks after discontinuation of the sulfonylurea therapy. However, this patient also received numerous other drugs, several of which have been reported to cause hemolytic anemia occasionally.

Endocrine system: Based on evaluation of routine autopsy slides there appeared to be a moderate increase in pituitary basophils in cases 15, 17 and 18, and a moderate increase in pituitary eosinophils in case 2. Two infarcts of the pituitary, cases 12 and 16, were no doubt related to cardiovascular disease which led to vascular collapse. There was no clinical or laboratory evidence of pituitary abnormality in any of the sulfonylurea-treated patients here described.

There are numerous reports of the production of goiter in animals by the administration of the sulfonylureas.[21,22,29,43,44] In some patients the sulfonylureas have been reported to depress the basal metabolic rate and the radioactive iodine uptake of the thyroid.[45–48] The present study revealed no evidence of clinical, laboratory or histologic abnormalities of the thyroid produced by the sulfonylureas. Colloid goiters present in three cases (5, 11, and 15) were observed prior to starting the sulfonylurea therapy and were not influenced by the drug.

The adrenal cortex was hyperplastic in 71 per cent of the sulfonylurea-treated patients comprising this report, but in only 56 per cent of the diabetic control series. The diagnosis of hyperplasia was made whenever the combined weight of both adrenal glands exceeded thirteen grams. The increased weight was due to a nodular hyperplasia of zona fasciculata and zona reticularis.

There was no other clinical, laboratory or histologic evidence of adrenal alteration. Vacuolization of the adrenal medullary chromaffin cells previously reported in rabbits[1] was present in approximately 50 per cent of the sulfonylurea-treated patients, but this finding is considered nonspecific since it is found so often in other conditions and in all cases showing appreciable adrenal autolysis. The adrenal microgranulomata present in 47

per cent of the sulfonylurea-treated patients were identical with those seen in other organs. However, these adrenal lesions must be evaluated with caution since cellular infiltrates, difficult to differentiate from microgranulomata, are very common in the adrenal. Such cellular infiltrates of the adrenal were also present in 40 per cent of the diabetic control series.

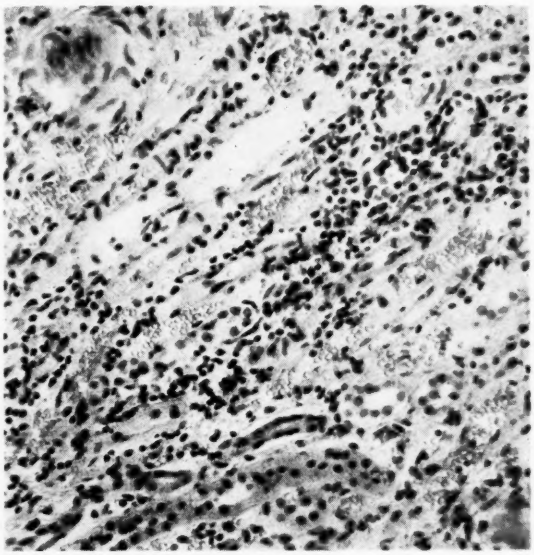

FIG. 7. Granulomatous interstitial nephritis, case 6. There is a diffuse cellular infiltrate in the medulla with atrophy and occasionally destruction of tubules. Hematoxylin and eosin X 210.

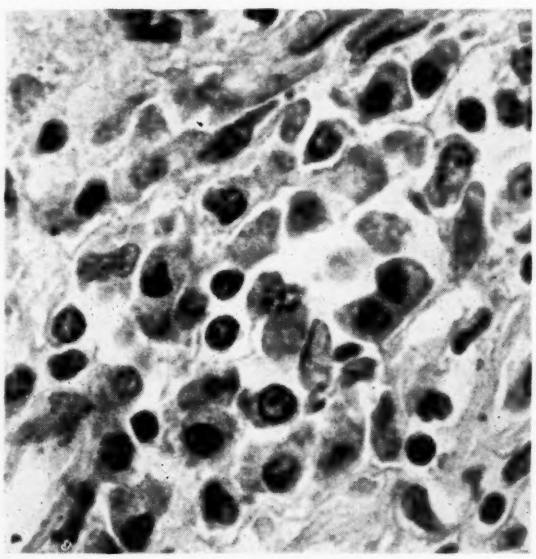

FIG. 8. Granulomatous interstitial nephritis, case 6. A higher magnification of figure 7 shows the lymphocytes, plasma cells and acidophilic histiocytes, typical of the cellular infiltrate. Hematoxylin and eosin X 1,040.

Kidney: Microgranulomata were found in 71 per cent of the patients receiving sulfonylurea drugs, but in only 30 per cent of the diabetic controls. This is a significant difference despite the difficulties in interpretation occasioned by the frequent occurrence of chronic pyelonephritis. The microgranulomata could be differentiated from the cellular infiltrates of pyelonephritis by the circumscribed, "punched out" appearance of the microgranulomata, the predominance of acidophilic histiocytes and the frequent presence of eosinophils.

Granulomatous interstitial nephritis was present in three patients, cases 4, 5, and 6 (figures 7, 8). This lesion consists of focal, although poorly circumscribed, infiltrates of cells of the same types comprising the microgranulomata. The cellular infiltrates usually surround fifteen to twenty medullary tubules, some of which show focal necrosis. Identical lesions were described in the kidneys of patients receiving sulfonamides.[5] One report of a similar lesion in a patient receiving carbutamide has appeared.[4]

The administration of sulfonylurea drugs has produced crystalluria, renal tubular degeneration and proteinuria in animals.[1,21,24,30]

There is one report of progressive diabetic nephropathy with uremia,[36] and one report of nephrosis[49] in patients receiving sulfonylurea drugs. In both cases the authors have assumed a causal relationship to the drug. These previously reported renal changes were not found in the sulfonylurea-treated patients comprising this report. All instances of azotemia and uremia encountered in our patients were due to pre-existing renal disease or cardiac failure; no clinical or laboratory evidence of abnormal renal function due to the sulfonylureas was found. Diabetic glomerulosclerosis was present in 71 per cent of the sulfonylurea-treated patients. It should be noted that this is a higher incidence of diabetic glomerulosclerosis than usually reported. This may be due to the inclusion of the diffuse form of diabetic glomerulosclerosis in our statistics and the examination of multiple sections of each kidney. Occasional foci of bacterial pyelonephritis were also encountered.

Nervous system: Hypoglycemia with coma, convulsions and death can be produced by exceeding the therapeutic level of sulfonylurea administration especially if food intake is inadequate.[29] Such deaths are usually unaccompanied by specific pathologic lesions of the nervous system. Agitation, weakness, drowsiness, tremor and con-

vulsions with death in the absence of hypoglycemia have been produced in animals by acute overdosage with the sulfonylureas.[1,21,22] The administration of sulfonylureas to patients[5,6,51-53] has been followed by rare instances of headache, drowsiness, paresthesia, mental confusion, seventh nerve palsy, exacerbation of paralysis agitans and coma. Histopathologic changes in the nervous system have not been reported following administration of sulfonylureas to either animals or man.[4,20]

The brain was examined in sixteen of the seventeen patients receiving sulfonylurea therapy at the time of death and, of these, five had evidence of a cerebral vascular accident, an incidence of 31 per cent. This is approximately the same incidence as that found in the diabetic controls, 37 per cent. There were also three patients with diffuse brain atrophy, cases 3, 4, and 15, an incidence of 19 per cent. The incidence of brain atrophy in the diabetic controls was the same, 19 per cent. Thus the data obtained in this study gave no clinical, laboratory or pathologic evidence of nervous system changes attributable to the administration of the sulfonylurea drugs.

CONCLUSIONS

Granulomatous lesions were found in a significantly higher percentage of sulfonylurea-treated patients than in diabetic controls examined in the same manner. Similar granulomatous lesions may be found in other conditions, therefore they are not specific. These lesions are similar to the granulomatous lesions which were reported in patients treated with the sulfonamides and assumed to represent a hypersensitivity reaction. The granulomatous lesions occur just as frequently after short-term therapy as after extended administration of the sulfonylureas. Therefore, one may assume that the reactivity of the individual patient is more important than the duration of administration or total dosage of the sulfonylurea drug. This finding is compatible with the concept that these lesions are due to hypersensitivity.

There was no correlation between the granulomatous lesions and clinical symptoms, laboratory abnormalities or cause of death. All clinical symptoms, laboratory abnormalities and deaths could be explained by other pathologic conditions found at autopsy. Two patients, cases 1 and 3, died suddenly and myocarditis was found in both hearts. However, other possibly fatal lesions were also present. Nonetheless, the high incidence of myocarditis, a potentially fatal condition, indicates the need for careful clinical and postmortem evaluation of the heart.

Examination of the pancreas did not reveal a correlation between pancreatic pathology and the clinical response of the patient to the drug.

SUMMARY

Twenty autopsied cases of diabetes mellitus treated with the sulfonylurea drugs have been reported. An incidence of myocarditis and microgranulomata higher than in diabetic controls was found. These lesions, which resemble the changes reported after sulfonamide administration, were apparently asymptomatic. It is suggested that these lesions represent a hypersensitivity reaction. No other untoward effects of the sulfonylurea drugs were found.

SUMMARIO IN INTERLINGUA

Lesiones Histopathologic, Associate con le Administration de Sulfonylurea

Es reportate vinti necropsias in patientes de diabete mellite tractate con le drogas sulfonylureal. Esseva trovate un incidentia de myocarditis e de microgranulomas plus alte que in un gruppo de controlo de altere diabeticos. Le lesiones—simile in apparentia al alterationes reportate post le administration de sulfonamida—esseva apparentemente asymptomatic. Es proponite le these que iste lesiones representa un reaction de hypersensibilitate. Nulle altere effectos adverse del sulfonylureas esseva constatate.

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Glucose Tolerance Curves in Neuropsychiatric Patients

Abraham M. Balter, M.D., Lyons, New Jersey

Mass survey is the most effective way of determining among psychotics the incidence of pathologic conditions, particularly those in which symptoms are ordinarily absent. Diabetes is an outstanding example.

Although a combination of the two-hour postprandial urinalysis and blood sugar determination is the usual preliminary to the study of large groups of people for the detection of diabetes,[1,7,8] the problems of management of psychotic patients for these tests involve much error. Furthermore, if either or both tests are positive, the entire procedure of handling patients must be repeated for the glucose tolerance test. Not much more handling of patients is required to make this test in the first place and the yield of information is much greater.

A study was undertaken, therefore, without preliminary screening on a ward in which the average age was sixty-one years. The incidence of abnormal tests was so high in this age group that the patients in several additional wards were examined in order to obtain a more accurate sampling of all age groups in the hospital. Of a total of 2,263 patients, 767 were given a glucose tolerance test.

MATERIALS AND METHODS

The hospital population is composed of two large groups of veterans: (1) those of the Korean War and World War II, ranging in age from twenty-six to eighty-one years, and (2) those of World War I and the Spanish American War, ranging in age from fifty-six to ninety-two years. A small number are peacetime veterans who served during the period between the two world wars and after the Korean War.

No special dietary preparations were made previous to the test, but the patients were allowed to eat their regular diet, which averages about 2,800 calories per day, and contains about 300 gm. of carbohydrate.

After a fasting blood sugar sample, the patient was given 100 gm. of glucose in water by mouth, and samples of venous blood were taken one-half, one, two and three hours afterward. Determinations were made by the Folin-Wu method. Each time blood was drawn, the patient emptied his bladder and the urine was tested for glucose by the Tes-Tape method.

Less than one tenth of 1 per cent was disregarded and the urine was considered normal. The limit for normal fasting blood sugar level was established at 120 mg. per 100 ml., the normal peak level following the ingestion of the test dose of glucose at 180 mg. per 100 ml., and the two-hour level at 120 mg. per 100 ml. These levels serve only for purposes of comparison.

The 767 patients were divided by decades into five age groups. Because there were only twenty-four patients whose ages ranged from seventy to eighty-four years, they were included in the group of sixty to sixty-nine years. All weights that were 5 per cent above or below standard* were included in the normal group.

Liver function tests were not made with the glucose tolerance tests. However, since March 1956, 2,576 patients were given liver-function tests preliminary to treatment with tranquilizing drugs. The cephalin flocculation, thymol turbidity and serum bilirubin tests were used. The results of the tests were studied for possible relationship to the incidence of diabetes.

FINDINGS

The 767 glucose tolerance curves were divided into three main divisions: (1) curves with a peak level higher than 180 mg. per 100 ml.; (2) curves with a peak level of 121-180 mg. per 100 ml.; (3) flat curves in which the peak blood sugar level did not rise above 120 mg. per 100 ml.

Group 1—Peak blood sugar level above 180 mg. per 100 ml. Of the 767 patients, 233 or 30.4 per cent had a glucose tolerance curve with peak levels above 180 mg. per 100 ml. The incidence of so-called diabetes-type curves depends on the height at which the critical peak blood sugar level is placed. If the critical level were to be raised by 25 mg. gradations, the incidence would fall from 30.4 per cent for peak levels above

From the U.S. Veterans Administration Hospital, Lyons, New Jersey.

*Association of Life Insurance Medical Directors of America, Vol. I, 1912-1914.

TABLE 1

Group I. This shows the proportion of the 767 patients who would be regarded as diabetic if the critical peak level were raised by 25 mg. gradations from a "normal" peak level of 180 mg. per 100 ml.

Total number examined	Above 180 mg.		Above 200 mg.		Above 225 mg.		Above 250 mg.		Above 275 mg.		Above 300 mg.	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
767	233	30.4	135	17.6	84	11.0	35	4.6	23	3.0	13	1.7

180 mg. per 100 ml. to 1.7 per cent for levels above 300 mg. per 100 ml. (table 1).

Only 15 per cent of patients in Group 1 had peak blood sugar levels above 250 mg. per 100 ml. as compared with 85 per cent having peak blood sugar levels ranging from 121 to 250 mg. per 100 ml. The fasting blood sugar was above 120 mg. per 100 ml. in only twenty-four patients, or 3.1 per cent of the 767 who were tested. There was no consistent relationship between the height of the peak blood sugar levels and the incidence of fasting blood sugars above 120 mg. per 100 ml.

Urinalyses made during the glucose tolerance tests showed that the largest proportion of glycosurics was found among those having the higher peak blood sugar levels. However, numerous patients having peak blood sugar levels no higher than 200 mg. per 100 ml. had glycosuria, while others having peak blood sugar levels as high as 275 mg. per 100 ml. were sugar free.

In the majority of cases in Group 1, the time of appearance of the peak blood sugar level was later than in the other groups. A major portion, 67.8 per cent, reached peak level after one hour, and 15.9 per cent after two hours, making a total of 83.7 per cent which reached peak level after one hour or more. Only 15.9 per cent reached peak level after one-half hour.

The height of the peak blood sugar levels in Group 1 bore no relationship to age. However, the incidence of the so-called diabetic curves, regardless of the height of the peak blood sugar level, bore a close relationship to age, increasing steadily with advance in years. In the age group of twenty to twenty-nine years it was 20.9 per cent as compared to 52.8 per cent in the age group of sixty to eighty-four years (figure 1).

Among the patients in Group 1, 172 or 73.5 per cent had two-hour blood sugar levels of 135 mg. per 100 ml. or above. Even among those whose peak blood sugar level rose no higher than 200 mg. per 100 ml., 50 per cent had two-hour blood sugar levels ranging between 145-200 mg. per 100 ml., and 19.4 per cent had three-hour levels ranging between 135-175 mg. per 100 ml. A consistent relationship was found between the height of the peak blood sugar levels, and the two-hour levels. The higher the peak blood sugar level the higher was the two-hour level and the greater was the proportion of patients having two-hour levels of 135 mg. per 100 ml. or above.

Patients of all types having three-hour blood sugar levels of 135 mg. per 100 ml. or above comprised a group of sixty-one or 8 per cent of the 767 patients examined.

Group 2—Curves with peak level of 121 to 180 mg. per 100 ml. This group comprised 452 subjects of which sixty-three or 13.9 per cent had two-hour blood sugars ranging between 135-175 mg. per 100 ml., fifty-three or 11.7 per cent had glycosuria, and thirteen or 2.9 per cent had both glycosuria and two-hour blood sugars ranging between 140 to 170 mg. per 100 ml.

Group 3—Flat curves. There were eighty-two flat curves or 10.7 per cent of the total. These did not go above 120 mg. per 100 ml. during the entire three hours of the test. Flat curves were found with diminishing frequency with advance in age.

Weight. Both overweight and underweight are problems in this hospital, but overweight is the greater of the two. Three hundred and nineteen of the 767 patients were 6 per cent or more overweight. When the number of overweight patients in each of the three groups was compared to the total number in each group, the subjects in Group 1 had the largest proportion of overweight patients, 45.5 per cent. The lowest proportion, 27.9 per cent, was found in the group having flat curves in which was also found the largest proportion of younger patients and the largest proportion of those who were underweight (table 2).

The extent of obesity or underweight in individual patients occurred largely within a range of 6 to 25 per cent, but beyond this level the proportion of overweight patients was much higher than that of the underweight patients. Thus of the 319 overweight patients, 221 or 69.3 per cent were overweight, 6 to 25 per cent, and ninety-eight or 30.7 per cent were overweight above 25 per cent. Among the 234 underweight patients, however, 212 or 90.6 per cent were underweight from 6 to 25 per cent, and only twenty-two or 9.4 per cent were underweight beyond this level.

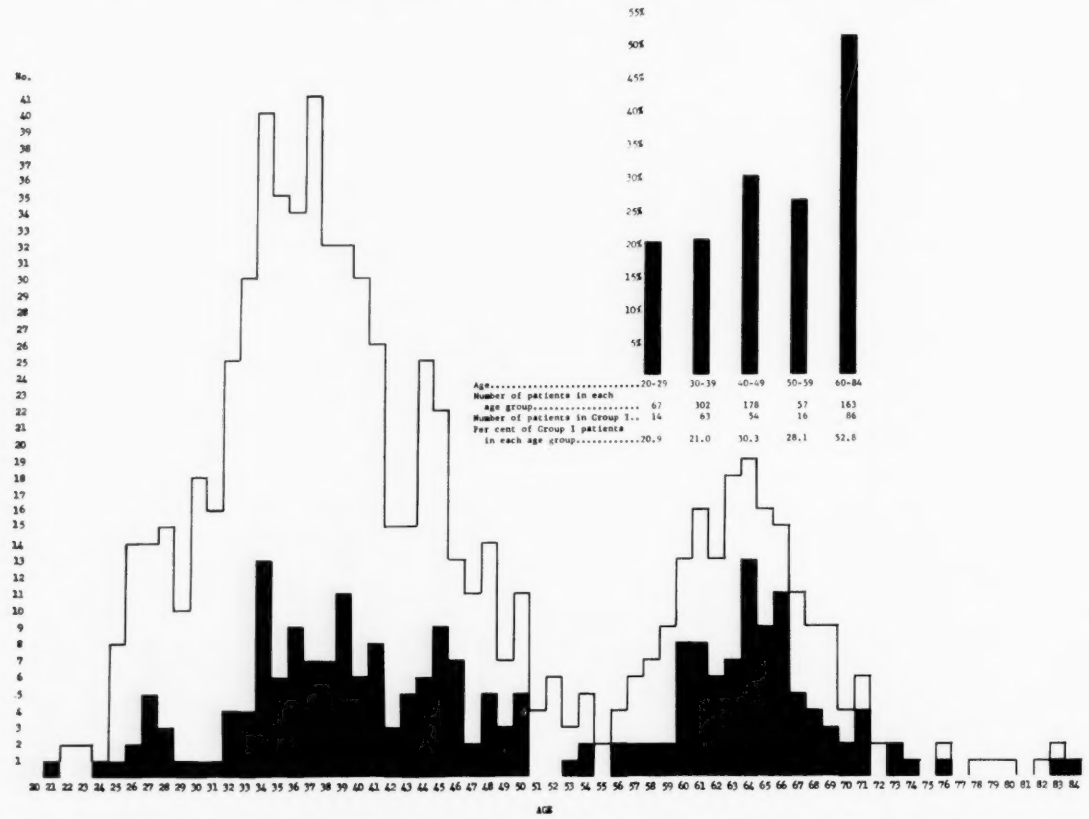

FIG. 1. The area in outline represents all the patients who received glucose tolerance tests. The area in black represents those who had a peak blood sugar above 180 mg. per 100 ml. The percentage scale shows that the incidence of diabetic type of responses increases directly with age.

TABLE 2

Comparison of number and per cent of patients in the three groups who were of normal weight plus or minus 5 per cent, with those who were overweight or underweight 6 per cent or more. The incidence of overweight, underweight and normal weight is shown in each of the three groups of response curves.

	Group I		Group II		Group III		Total	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Overweight 6 per cent or more	106	45.5	190	42.0	23	28.0	319	41.6
Underweight 6 per cent or more	65	28.0	140	31.0	29	35.4	234	30.5
Normal	62	26.5	122	26.9	30	36.6	214	27.9
Total	233	100.0	452	100.0	82	100.0	767	100.0

The relationship between response to the glucose tolerance test and weight was explored. For each combination of glucose tolerance curve and weight, the number of cases found among the 767 patients was compared to the number of cases to be expected if these two variables had only a chance relationship. Although there was some tendency in the direction of a positive relationship, it was not significantly different from chance.

A significant difference was found, however, with re-

spect to the incidence of overweight, underweight and normal weight among patients having flat glucose tolerance curves. Overweight patients in table 2 were relatively infrequent in the flat curve group.

A further approach to this problem was to examine the incidence of Group 1 subjects among those of normal weight, moderately overweight and grossly overweight patients. No significant difference was found between degree of overweight and type of glucose tolerance curve.

Liver Disease: Between April 1, 1956, and March 16, 1959, 2,576 patients were tested for liver function by the cephalin flocculation, thymol turbidity and serum bilirubin tests. Among these were 438 patients who had also received a glucose tolerance test. No parallel could be found between the abnormal liver function test and the abnormal glucose tolerance test. Many patients with abnormal liver function tests had normal glucose tolerance tests, and many with abnormal glucose tolerance tests had normal liver function tests.

DISCUSSION

The present study was made without preliminary screening; 767 patients ranging in age from twenty to eighty-four years were given a glucose tolerance test. Regardless of the criteria that might be established for the diagnosis of the diabetic type, the response to the glucose tolerance test was found to be a continuous process running from the normal to the pathologic. No line of demarcation could be found between the unquestionable diabetic and the equally unquestionable nondiabetic. Neither the peak blood sugar level, nor the time of its appearance, nor the two-hour blood sugar level, nor glycosuria, nor all combined could demonstrate where the diabetic condition ceased and the nondiabetic began. This is consistent with a recent statement by Best[3] regarding the potentiality for diabetes of all people, if a sufficiently sensitive test could be devised. The fine gradations through which the overt diabetics merge with the mild diabetics, and these with the potential diabetics, make necessary an arbitrary definition of the point at which diabetes is indicated by the glucose tolerance test. This was clearly shown in the present study in which the critical peak blood sugar level was set at 180 mg. per 100 ml. Of the 767 patients, 233 or 30.4 per cent could be placed in the so-called diabetic group, but if the critical peak blood sugar level were set at 200 mg. per 100 ml., which is the criterion in current use,[2] only 17.6 per cent of the patients could be described as "diabetic."

Of all the criteria for the prediction of the glucose tolerance response, the fasting blood sugar was least reliable. A high fasting blood sugar could be found among the very mild types of so-called diabetics, while it could be absent in patients whose response to the glucose tolerance test was well within this range.

Although the great majority of patients are symptomless and of the so-called mild or potential type, they must always be regarded with suspicion even though they may never reach the point of clinical diabetes. Their importance was demonstrated by the Oxford[4] study in which it was shown that, after a four-year follow-up, the suspicious cases which were not at first described as diabetic later produced eight times as much diabetes as did those who had none of the stigmata of diabetes. It is possible that this tendency toward overt diabetes is indicated in the so-called mild and potential diabetics by the close relationship that was found between the peak blood sugar level, the time of its appearance, the two-hour blood sugar level and glycosuria. In the great majority of cases, a high peak blood sugar level tended to appear late, and was accompanied by a high two-hour level and glycosuria.

The increased incidence of abnormal glucose tolerance curves with age only indicates one aspect of the general reduction in metabolic efficiency that takes place during the aging process. Many different patterns of deterioration can be found in neuropsychiatric institutions where patients can be observed over long periods of time.

Obesity was not found significantly more often among the subjects with abnormal responses to the glucose tolerance test than among those with normal responses. There was a slight difference in the incidence of obesity between the subjects having a high curve and those having flat curves. However, age may account for this because the majority of subjects having flat curves were in the younger age groups. It would appear that obesity is not the cause of an unusual response to the glucose tolerance test; it is rather a deviation from the normal state that would tend to disturb the metabolic efficiency of the body by placing an unusual strain upon it, thus creating a stress under which certain metabolic patterns would tend to fail more readily than others.

At the present time the application of these findings to the general population is not warranted. It must be admitted that in certain respects psychotics differ from nonpsychotics. In this hospital, typical hay fever is quite rare; colitis and acute rheumatoid arthritis are unknown; peptic ulcer is common; and myocardial infarction is very frequent. Nevertheless the high incidence of abnormal responses calls into question the findings made in mass surveys of the general population in which the

incidence of diabetes has been found to range from 1.2 per cent[5] to 4 per cent.[6] It is significant that Petrie and his associates[7] found that the prevalence of diabetes increased directly with age, and the highest incidence, 7.38 per cent, was found in people over seventy years of age.

It is unknown how the incidence of diabetes in this hospital would compare with that of the general population because this investigation has the handicap of having been made without a control group of an equal number of nonpsychotics of the same age and sex. Furthermore, no large body of information exists concerning the response to the glucose tolerance test of large and unscreened numbers of people comparable to that of tuberculosis case findings in which many thousands of people have had roentgenograms of the chest without preliminary screening by history, physical examination or tuberculin test.

In view of the fact that testing all patients in this hospital without preliminary screening increased the number of patients who might be classed as diabetic from 3.1 per cent to 30.4 per cent, the impression is very strong that the usual methods of detection of diabetes in the general population are probably inadequate for the discovery of an unknown but considerable number of moderate and mild diabetics. More important, they may fail to find the great majority of potential diabetics, and thus, for many people, the advantages of early diagnosis and treatment are lost.

SUMMARY

1. The testing of 767 patients in this hospital with the glucose tolerance test without preliminary screening showed that 30.4 per cent of the patients could be classed as diabetics if the critical peak blood sugar level were set at 180 mg. per 100 ml. However, the gradations from overt diabetes to mild, and potential and nondiabetes were so gradual that no dividing line could be established between diabetics and nondiabetics.

2. A definite relationship was found between age and abnormal response to the glucose tolerance test.

3. A distinct relationship between overweight and abnormal response to the glucose tolerance test was not found.

4. At the present time the findings among psychotics may not be applied to the general population because no large body of people has been tested with the glucose tolerance test without preliminary screening. The impression remains, however, that the incidence of diabetes in the general population is probably much greater than is generally believed.

SUMMARIO IN INTERLINGUA

Curvas del Tolerantia a Glucosa in Patientes Neuropsychiatric

1. Le test del tolerantia a glucosa in 767 patientes de iste hospital monstrava—sin previe selection—que 30,4 pro cento del casos poteva esser classate como diabetic super le base de un nivello critic pro le maximo de sucro sanguinee de 180 mg per 100 ml. Tamen, le gradationes ab diabete patente a diabete leve e potential a non-diabete esseva si continue que il esseva impossibile establir un linea de division inter diabeticos e non-diabeticos.

2. Esseva constatate un definite relation inter le etate del patientes e le anormalitate de lor responsa in le test de tolerantia a glucosa.

3. Non esseva trovate un relation distincte inter excesso de peso e anormalitate de responsa in le test de tolerantia a glucosa.

4. Al tempore presente, le constatationes inter un population de psychoticos non pote esser applicate al population in general, proque le test del tolerantia a glucosa ha nunquam essite applicate a un grande numero de subjectos sin selection preliminari. Remane, nonobstante, le impression que le incidentia de diabete in le population in general es probabilemente multo plus alte que lo que es usualmente supponite.

ACKNOWLEDGMENT

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Anaphylactic Shock Following Insulin Injection

Case Report and Review of the Literature

Lonnie Hanauer, A.B., and Jack M. Batson, M.D., New York

Allergic reactions following the use of insulin, while not uncommon, are rarely severe. A severe reaction when it does occur involves an acute life-threatening situation, as well as with the continuing problem of treating the diabetic state. Recent experience with one such case led to a review of the literature in English where seven cases of anaphylaxis following insulin injections were found. Three of these occurred in diabetics, none of whom was in ketosis. The purpose of this report is to present a case of anaphylaxis following insulin administration for diabetic ketosis, and to review briefly the problem of insulin allergy.

The over-all incidence of allergic reactions of any type following insulin injection is between 5 and 10 per cent.[1,2] Such reactions may be divided into three types:[3] mild local, severe local, and generalized. Eighty-five per cent of insulin reactions are of the mild, localized type with stinging, redness, and induration at the injection site, reaching a maximum in twelve to twenty-four hours and subsiding in one to three days. These reactions usually clear in spite of continued use of insulin, but they occasionally become worse, and they may even become generalized.[4,5] It has been shown that the frequency and severity of mild local reactions are just as great in non-diabetic controls receiving insulin for the first time as in regular insulin users.[6]

About 15 per cent of insulin reactions are more severe, causing extreme pain, marked induration, urticaria, and erythema, localized to the injection site. Maculopapular rash,[7] large local bullae,[8] and localized purpura without thrombocytopenia[9] are among the severe local reactions that have been reported.

Only 1 to 2 per cent of reactions to insulin give systemic manifestations. Yasuna[10] reviewed the literature prior to 1941 and found eleven cases, to which he added another, in which generalized sensitivity to insulin was verified by means of skin tests. Generalized manifestations are usually cutaneous, circulatory, and/or gastrointestinal. The cutaneous reactions are most common,[1,11–16] and include urticaria, swelling, rash, pruritis, and paresthesias. The circulatory reactions include pallor, flushing, palpitation, and circulatory collapse.[10,17] Nausea, vomiting, abdominal cramps, and diarrhea are among the gastrointestinal symptoms.[1,8,13,17]

Anaphylactic shock is the least common of the allergic reactions to insulin. Seven such cases were found in the literature in English, and these, along with the case presented here, are reviewed in table I. This case is apparently the first reported with anaphylactic shock from insulin allergy occurring in a patient with diabetic ketosis.

CASE REPORT

A.F., a fifty-eight-year-old female Negro, was first seen at the New York Hospital on July 19, 1957, complaining of vaginal pruritis of two months' duration. She gave a history of polydipsia, dry mouth, nocturia (six to seven times a night), frequency and urgency of urination, and hypertension. She had five normal full-term spontaneous deliveries from 1921 to 1927; the second infant weighed twelve pounds, but all of the others were under seven pounds at birth. Her urine had recently been tested for sugar with negative results.

Physical examination revealed an extremely obese Negro woman, 165 cm. in height, and 102.3 kg. in weight. The pulse rate was 125 per minute, and the blood pressure was 155/110 mm. Hg. Crepitant rales were present at both lung bases. The vulva had a whitish tint. Urine glucose determination was three plus.

Four days later she was seen in the general medical clinic, and additional complaints of malaise, fatigue, anorexia, and a thirty-six-pound weight loss in the previous three months were elicited. There was no family history of diabetes.

The urine gave a four-plus reaction for sugar, and a strongly positive reaction for acetone. Hemoglobin, white blood cell count, and differential count were all normal. The blood sugar was 524 mg. per 100 ml., the carbon dioxide combining power, 23 mm. per liter, and the blood urea nitrogen, 7 mg. per 100 ml. Serum acetone was negative.

Winners of honorable mention in the 1959-60 Graduate and Medical Student-Intern Essay Contest of the American Diabetes Association for the best review article or case report. This is the paper for which they received their award.

From the Department of Medicine, The New York Hospital-Cornell University Medical Center, New York City. Mr. Hanauer's present address is: The Beth Israel Hospital (Med.), Boston, Massachusetts. Dr. Batson's present address is: Vanderbilt University Hospital, Nashville, Tennessee.

The patient was admitted to the hospital where the ketosis was corrected with Regular Insulin. The patient was then given 40 units of NPH insulin per day with adequate control.

When seen in the clinic two weeks after discharge, she reported a few "lumps" at the sites of her insulin injections. The blood sugar was 97 mg. per 100 ml. Over the next two months the amount of insulin was reduced gradually, and finally discontinued.

The patient failed to return until July 14, 1959, when she complained of polyuria and polydipsia of two months' duration. Urine tests done at home were positive for sugar, and on one occasion, for acetone. Six weeks prior to admission NPH insulin was again administered by her local physician at a dose of 40 units daily. One week later pruritic "lumps" appeared over much of her body. Insulin was withheld, and the "lumps" disappeared, but her weakness, polyuria, and polydipsia increased.

Physical examination at this time showed her weight to be 109 kg., and her blood pressure, 200/130 mm. Hg. Funduscopic examination revealed Grade II hypertensive changes. There was slightly decreased sensation to pain and light touch in the lower extremities. The remainder of the physical examination was normal. A weight reduction diet was reinstituted.

Three weeks later she returned with progressive weakness and extreme polyuria and polydipsia. She had had continuous four-plus urine sugar tests and strongly positive urine acetone tests for one week. She had lost 5 kg., apparently from dehydration.

Urinalysis revealed a four-plus sugar reaction, strongly positive acetone, two-plus albumin, and a sediment containing four to eight white blood cells, three to five red blood cells, and zero to two coarse granular casts per high power field. Blood counts were normal. Blood urea nitrogen was 19 mg. per 100 ml.; blood sugar, 424 mg. per 100 ml.; carbon dioxide combining power, 23 mm. per liter; sodium, 129 mEq. per liter, and potassium, 4.3 mEq. per liter. After intravenous saline was started, the patient received 50 units of Regular Insulin intramuscularly followed immediately by 20 units intravenously. Four minutes later, the patient, who had previously been alert and talkative, became unresponsive and collapsed. Her breathing became slow and deep. Her heart sounds were faint, and her blood pressure was unobtainable. Fifty cubic centimeters of 50 per cent dextrose in water was given without effect. Two cubic centimeters of 1:1,000 epinephrin given intravenously led to rapid recovery.

Beginning at 1:30 a.m. she slowly was desensitized to insulin, (method of Corcoran[18]), first receiving 0.001 unit of Regular Insulin subcutaneously, and then increasing amounts throughout the day until 4:15 p.m. when 40 units was well tolerated. She also received hydrocortisone (300 mg. daily), tetracycline, streptomycin, and tolbutamide. Just before desensitization was complete the blood sugar was 640 mg. per 100 ml., and the carbon dioxide combining power was 20 mm. per liter. Serum acetone was negative. She subsequently received 200 units of Regular Insulin without allergic reaction, and the ketosis gradually cleared. After six days the insulin was discontinued, and tolbutamide was begun.

The remainder of the hospital course was complicated by a cerebral hemorrhage (two days after the anaphylactic episode), pneumonitis, and a urinary tract infection. She was discharged on the twenty-fifth hospital day, with her diabetes adequately controlled by a weight reduction diet and 1.5 gm. tolbutamide daily. She was well except for minimal residual weakness of her left arm.

One month later skin tests were done with the following results. A direct scratch test with undiluted U40 Regular Insulin was negative. Intracutaneous injection of 0.02 cc. of 1:100 dilution of U40 Regular Insulin (0.008 units) resulted in erythema and a one centimeter diameter wheal with small pseudopodia. Saline control was negative as was injection of 0.02 cc. of both beef and pork antigen, each containing 100 nitrogen units per cubic centimeter.

The patient has remained well with weight reduction, dietary regulation, and 1.0 gm. of tolbutamide per day.

REVIEW OF CASES

Seven cases of anaphylaxis following insulin injection were found in the literature in English,[19–25] only three in diabetics (table 1). All of the patients who had anaphylaxis had received insulin before. One had a history of eczema, three had no history of allergy, and in three this information is not available. All of these patients had hypotension and collapse. Four had cutaneous manifestations including urticaria, pruritis, and flushing. Four had pulmonary difficulties, including cough, bronchospasm, and dyspnea, and five had gastrointestinal disturbances including nausea, vomiting, and incontinence of stool. There were no deaths. Skin tests were done in six cases with positive results in all.

DISCUSSION

Reactions to the first impure insulins produced may have occurred in as many as one of three patients,[4] but by 1925 preparations were becoming more purified and reactions less frequent. Nevertheless, a large percentage of allergic reactions to insulin is probably caused by impurities.[26] Jorpes makes the statement that "true insulin allergy must be considered as extremely rare,"[27] and tells of one patient who could identify four insulin preparations in order of purity strictly by his increasing sensitivity to them.[4] Protamine has been found to be nonantigenic, and is thus eliminated as the allergic factor.[9] Due to the early success of Crystalline Insulin some authors believed that a completely pure insulin would be nonallergenic,[28] but others showed sensitivity to insulin from many sources,[29] and even to insulin which had been recrystallized twelve times and precipitated at the isoelectric point four times.[4]

Insulin is a complete protein and an active antigen capable of producing sensitization. Lewis[30] utilized the Schultz-Dale technic to study the antigenic properties of insulin. He found that in strips of uterus of animals sensitized to beef insulin, specific contractions were induced with beef insulin but not with beef pancreas or beef serum. Uteri of animals sensitized with either the beef or pork insulin responded to tests with the heterologous insulin. He concluded that insulin proteins

TABLE 1

Author	Age Sex	Indication for insulin	Interval from last insulin	Type of insulin	Amount (units)	Route	Interval to onset	Previous insulin allergy	Skin tests: Insulin	Skin tests: Animal protein	Miscellaneous
1. Rose and Barron[19]	40 M	Shock therapy	3 years	Soluble	160	I.V.	5 min.	None	Pos.	—	Passive transfer positive.
2. Hinko, Fenton, and Balberer[20]	30 M	Shock therapy	2 days	Soluble	105	Subcutaneous	20 min.	None	Pos.	Neg.	Passive transfer negative.
3. Benham[21]	35 F	Shock therapy	3 days	Regular	400	I.M.	30 min.	None	—	—	—
4. Ulrich, Hooker, and Smith[22]	53 M	Underweight	7 years	Regular	5	?	few min.	None	Pos.	Neg.	Less severe reaction to insulin four months later. Subsequently desensitized. Passive transfer negative.
5. Watson[23]	58 F	Diabetes 15 years	3 years	Crystalline Zinc	8	?	5 min.	"Shock" one year earlier	Pos.	Neg.	Subsequently desensitized.
6. Wechsler, Farmer, and Urban[24]	65 F	Diabetes 7 years	1 year	Lente	20	?	45 min.	2 similar reactions previously	Pos.	Neg.	Anaphylaxis six weeks later after 3 units. Passive transfer inconclusive.
7. Lowell[25]	50 F	Diabetes 10 years	2 years	Regular	4	Intracutaneous	few min.	Urticaria	Pos.	—	Insulin resistance. Subsequently desensitized.
8. Hanauer and Batson	58 F	Diabetes 2 years	2 months	Regular	50 20	I.M. I.V.	4 min.	?Urticaria	Pos.	Neg.	Subsequently desensitized. Later given tolbutamide.

from different animal sources are immunologically closely related, though perhaps not identical. Tuft[2] had said earlier that insulin hypersensitivity could exist irrespective of the hormone's biologic source, and compared it to lens protein in this respect. Wasserman and Mirsky[31] sensitized guinea pigs to insulin and then induced anaphylaxis with insulin from a different species. They concluded that insulin derived from various animal sources was immunologically identical. Bernstein, Kirsner, and Turner[32] had previously caused anaphylaxis in guinea pigs sensitized with various insulin preparations, and similar work was recently reported by Moloney and Coval.[33]

Although an allergic reaction to insulin may have no effect on the hypoglycemic response,[26] the development of generalized insulin sensitivity may be accompanied by insulin resistance on the part of the patient, thereby necessitating larger doses of insulin for control of the diabetes. With disappearance of the sensitivity, insulin resistance also subsides.[34] Sherman[12] and Rudy[35] report cases of insulin-induced generalized urticaria accompanied by insulin resistance. Lowell[25] found evidence for two antibodies against Crystalline Insulin in one patient who was both allergic and resistant. There was an allergic antibody which was capable of passively transferring skin sensitivity and an insulin-neutralizing antibody which could destroy the physiologic effects of Crystalline Insulin. He found that the allergic antibody titer remained high for long periods, but that desensitization was possible. Then neutralizing antibody increased in titer during each course of therapy and resistance to insulin developed in ten to twelve days, then disappeared rapidly when treatment was stopped.

The apparent high incidence of insulin allergy in nondiabetics[9,19,21,22,32] might lead to the conclusion that it is more frequent in this group, and this had been suggested.[22] Bernstein[32] found that large groups of allergic individuals contained relatively few diabetics and suggested that the allergic and diabetic constitutions might be antithetic. Neither of these conclusions has been sustained. Kern[36] found a high incidence of allergic histories among diabetic patients although diabetes and allergy seldom occurred at the same time.

With insulin sensitivity, as with other allergic reactions, there is generally a lapse in exposure to the antigen preceding the severe allergic reaction to reexposure to the antigen. Goldner[37] reviewed fifteen cases of insulin allergy and found that nine had a lapse in therapy ranging from "months to years." He also found that allergy during the first course of treatment was

characterized by a seven to twenty-five day latent period. Seven of the eleven cases reviewed by Yasuna[10] had a known lapse in therapy, as did all of the cases reviewed here. Today many patients are being taken off insulin and placed on tolbutamide and other oral agents. A certain number of these patients will have to be returned to insulin therapy and in this group, the danger of insulin allergy and anaphylaxis becomes real.

Skin tests generally were used to confirm the diagnosis of insulin allergy, but Ljung[1] states that positive skin tests do not necessarily indicate that the patient will react in an allergic manner. Also, troublesome local reactions may occur with negative skin tests.[6]

Several methods have been used for treating insulin allergy. Rapid, reliable treatment is of the utmost importance when a patient is seriously ill and in need of insulin. Jorpes[4] found that insulin recrystallized three to five times was effective in preventing allergic reactions, but most of his patients were probably reacting to impurities rather than the insulin itself. Two patients who had had generalized allergic cutaneous reactions from insulin were successfully treated with Regular Insulin that had been placed in boiling water for thirty minutes. There was only a 10 to 20 per cent decrease in the hypoglycemic effect.[15,16] The value of antihistaminic drugs is debated. Gastineau and Leavitt[5] found diphenhydramine effective in controlling local and generalized urticaria, and Poliakoff[20] used the same drug, along with corticotropin, to prevent reaction in a patient with known insulin sensitivity. Rose and Barron[19] observed that mepyramine maleate injected along with insulin prevented a positive skin reaction. Dolger,[16] on the other hand, found that generalized allergic reactions to insulin responded unsatisfactorily to oral or parenteral antihistamines. Corcoran[18] desensitized a patient who had proved to be sensitive to recrystallized insulin. He started with 0.2 units commercial insulin injected intracutaneously, and increased the dose gradually until it reached 30 units intravenously within fifteen hours. Three of the patients reviewed by Yasuna[10] were successfully desensitized, as were the case reported here, three of the cases reviewed above,[22,23,25] and a number of other cases.[11,13,14]

SUMMARY

A case of anaphylactic shock following the injection of Regular Insulin in the treatment of diabetic ketosis is reported. Seven cases of anaphylaxis following insulin administration found in the literature in English are reviewed. The problems related to allergic reactions to insulin are discussed.

SUMMARIO IN INTERLINGUA

Choc Anaphylactic Post Injection de Insulina: Reporto de un Caso e Revista del Litteratura

Es reportate un caso de choc anaphylactic post le injection de Insulina Regular in le tractamento de cetosis diabetic. Es revistate le septe casos de anaphylaxe post administration de insulina que es trovate in le litteratura de lingua anglese. Le problemas relationate a reactiones allergic a insulina es discutite.

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Fatal Reactions to Drugs

Deaths from therapeutic misadventure are the subject of some instructive statistics in the latest annual commentary to come from the Registrar-General for England and Wales. In the two years 1957-58 there were 265 deaths due to adverse reactions to drugs or therapy. The deaths attributed to "therapy" comprised thirty-nine from X radiation, twenty from blood transfusion, and eleven from electroconvulsive treatment. Prominent among the drugs causing death was chloramphenicol, which continued to show its lethal potentialities in causing thirteen deaths; the causes were blood dyscrasias and in one case a hypersensitivity reaction. Chlorpromazine on its own caused ten deaths, in conjunction with reserpine one, and with pecazine one. In addition pecazine by itself caused three deaths. Anticoagulants caused death in nine cases plus another two when administered with other drugs; as would be expected, most of these were due to hemorrhage. The antirheumatic drug phenylbutazone was responsible for five deaths when given on its own, for one in conjunction with meprobamate, one with gold, and one with gold and salicylates. Gold itself caused four deaths. The sixteen deaths attributed to insulin included fourteen in coma; the other two were from acute heart failure and pulmonary edema. The sulfonamides were another group causing several deaths, there being ten cases in this category. The eight deaths caused by penicillin were all due to hypersensitivity reactions. Among the many other types of drugs causing fatal reactions were steroid hormones, antithyroid drugs, iron preparations, aspirin, mersalyl, and cytotoxic drugs. The commonest fatal response to a drug was blood dyscrasia, and after that a hypersensitivity reaction.

The previous inquiry of this nature covered three years instead of the two now reported on, so that the 302 deaths then recorded as due to therapeutic misadventure cannot be exactly compared with the 265 recorded now. But the present total does suggest that deaths of this kind are increasing in incidence. In general, much the same groups of drugs were found to be causing fatal reactions in the earlier inquiry—sulfonamides, penicillin, chloramphenicol, insulin, anticoagulants, phenylbutazone, steroids, antithyroid drugs, chlorpromazine. Obviously such drugs as these—among the most effectual in modern therapy—are often indispensable in the treatment of a patient. But their toxicity, and that of many other recently introduced drugs which also cause fatal reactions, needs emphasizing from time to time. It is interesting to note that the U.S. Food and Drug Administration has recently proposed regulations which would, among other things, draw the hazardous properties of a drug to the physician's attention by means of suitable wording on the label of its container.

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The Effect of Hypophyseal Stalk Section on Insulin Resistance

Richard A. Field, M.D., William A. Hall, M.D., William H. Baker, M.D., and Dagoberta Sosa, M.D., Boston

Four types of insulin antagonists have been described in the plasma of diabetic animals. One appears to be a beta lipoprotein which may be formed from, or under the influence of, somatotropin and an adrenal steroid.[1] Another, thought to be an alpha globulin, is present in the serum of insulin resistant patients during severe keto-acidosis, whether or not they had had prior treatment with heterologous insulin.[2] There is evidence that this factor may also be formed from, or under the influence of, somatotropin and adrenal steroid.[3] Vallance-Owen and Lilley have described an insulin antagonist residing in the albumin fraction from both uncontrolled insulin-requiring diabetics and normal human subjects, the activity of which is also dependent on the intact pituitary.[4] Lastly, Berson and Yalow have demonstrated an insulin-binding gamma globulin in the serum of all patients, diabetic or otherwise, after several months of heterologous insulin therapy.[5,6] The following case report is that of a patient who was suffering intractable pain from metastatic breast carcinoma and was subjected to hypophyseal stalk section in an effort to relieve her pain. Studies of the effect of this pituitary operation on her diabetes suggest that the latter immunologically stimulated antagonist may also be pituitary dependent.

CASE REPORT

(E.N. MGH #84898). This sixty-four-year-old housewife and mother entered the hospital in June 1959 because of severe pain from metastatic breast carcinoma. The patient had been massively obese for many years. In 1940, she underwent an uneventful menopause. In 1942, she had a subtotal thyroidectomy for a nodular goiter which had been present for many years. In 1943, a combined abdominal-perineal resection was performed for carcinoma of the rectum. No recurrence of this tumor has been found to date. In 1947, she developed diabetes mellitus, and in the following four years was well managed on diet alone, although she did not lose weight. Insulin was started in 1951, and for the following six years she was easily managed on 25 units of NPH insulin daily. In 1955, adenocarcinoma of the breast was discovered and she underwent left radical mastectomy. Early in 1957, insulin resistance appeared. At that time, there was no clinical evidence of tumor recurrence. She was found to be euthyroid and there was no evidence of a metabolic abnormality other than diabetes. In the ensuing twelve months, the dose of Crystalline Zinc Insulin was progressively increased to more than 300 units daily in order to free her from symptoms of florid diabetes. Late in 1957 she developed severe pain in the anterior chest. X rays of the chest and rib cage were unremarkable, but a biopsy of sternal marrow revealed metastatic carcinoma histologically consistent with origin from the breast lesion. A course of X-ray treatment to the involved ribs and sternum afforded no relief from the pain and had no effect on her insulin requirement.

In July 1959, the pituitary stalk was divided and a rubber diaphragm placed over the sella. During the operation and immediately afterward, several hundred milligrams of hydrocortisone were administered and, subsequently, she was given maintenance doses of 25 mg. daily. Postoperatively, there was complete relief of pain and to date there has been no recurrence of pain.

Pituitary gonadotropins, 17-ketosteroid and 17-hydroxysteroid excretion, protein-bound iodine levels and radioactive iodine uptake (performed at intervals after the operation) had declined, but remained within normal limits. Preoperative assay of insulin-antagonistic alpha globulins revealed measurable levels, but there were no significant or consistent changes postoperatively.

Assays of the patient's serum for insulin-binding gamma globulins were carried out just before operation and at intervals after operation. The patient's insulin:insulin-binding gamma globulin complex was of a very low energy of association. Most of Berson's insulin resistant patients have insulin antibodies which form two types of complexes, one of higher energy (12-13 kilocal./mol.), and one of lower energy (9-10 kilocal./mol.). This patient's serum showed complexes of binding energy which were below 9-10 kilocal./mol. This peculiarity of binding energy is unique in the extensive experience of Berson and Yalow, and tends to make precise quantitation of the insulin-binding gamma globulins somewhat difficult. However, the changes observed in this patient were of such a magnitude as to make this difficulty inconsequential.

Before operation, the insulin-binding gamma globulin level was more than 150 units/liter (figure 2). Ten days after

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From the Departments of Medicine and Neurosurgery, Harvard Medical School, and Massachusetts General Hospital, Boston, Massachusetts.

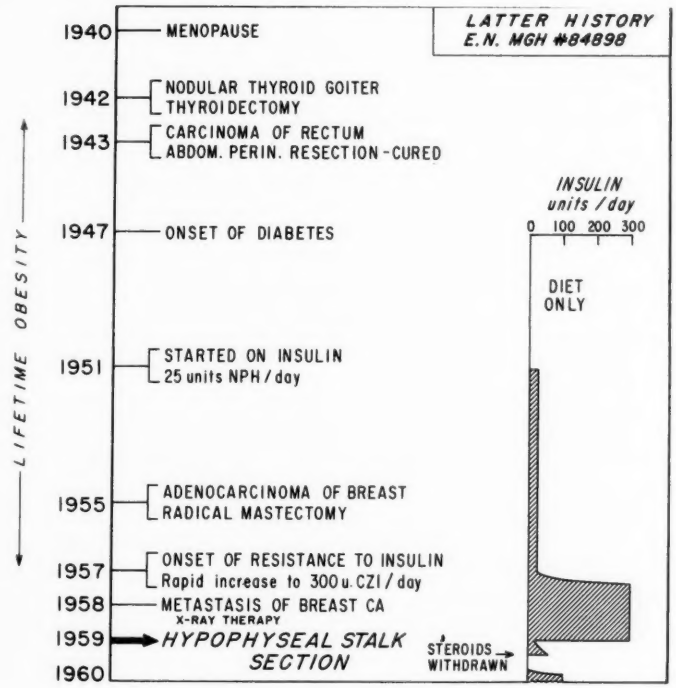

FIG. 1. Historical outline. Insulin requirement declined after operation and currently has stabilized at 27 per cent of the preoperative requirement.

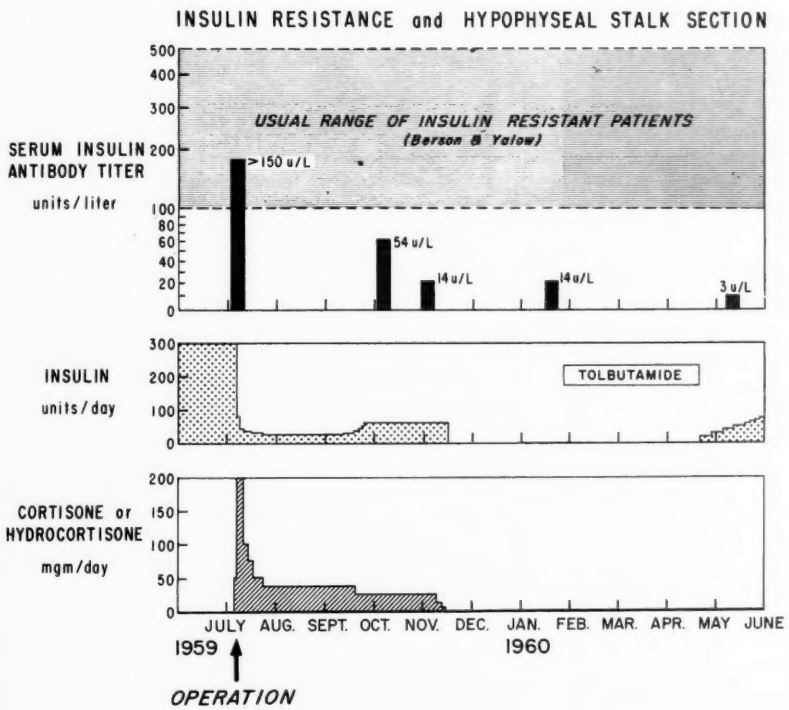

FIG. 2. Serum insulin-binding globulin level declines after operation, and is at lowest level eleven months postoperatively. Steroids were withdrawn 4½ months postoperatively.

operation, the level had decreased to 54 units/liter. After withdrawal of steroids four months later, the level was still further reduced to 14 units/liter. Most significantly, ten months after operation, despite the reinstitution of insulin three weeks earlier, the anti-insulin gamma globulin level was at its lowest, a definitely but barely measurable 3 units/liter.

Coincident with the initial postoperative fall in insulin antibodies, there was a clinical remission of diabetes accompanied by a diminished insulin "requirement" to 25 units of NPH insulin daily. During the four months following operation, there was a gradual increase in insulin requirement to 60 units of NPH insulin daily.

In early November 1959, steroids were withdrawn completely and the patient's fasting blood sugars promptly fell to normal and glycosuria disappeared. Administration of insulin was discontinued. For two months, without any specific therapy, the patient was clinically free of diabetes, but in January 1960, glycosuria and hyperglycemia recurred and tolbutamide was started. After an initial favorable response to tolbutamide, the effect of this agent gradually diminished, and in late April 1960, insulin was again started. With progressive increases in insulin to a current level of 80 units daily, the patient's blood sugars have been brought into a more physiological range and the symptoms of florid diabetes have subsided. Her weight has not fallen from the preoperative level of 180 lb. and she has regained her sense of well-being. She is active about her home and carries on her usual social activities.

DISCUSSION

The association of insulin resistance with insulin-binding gamma globulins has been clearly demonstrated by Berson and Yalow,[6,8] and others.[7,8] The potent effect of cortisone on reducing insulin resistance concomitant with reduction of insulin-binding antibodies has been noted by many workers, although Geller et al. have reported a case in which insulin resistance was precipitated by cortisone and reversed with nitrogen mustard.[9] BAL and ACTH are also known to suppress antibody formation, and Friedlander has successfully treated some insulin resistant patients with sulfonylurea agents.[7]

It is difficult to escape the conclusion that the reduction in insulin-binding globulin accompanying amelioration in diabetes in this patient had a cause-and-effect relationship. The following explanations are to be considered in accounting for the decline of antibody in this patient.

1. Cortisone replacement therapy in the postoperative period affected the antibody decline by suppressing its production at its source. This seems unlikely, since the insulin-binding globulin level continued to decline after steroids had been discontinued, reaching its lowest level six months later.

2. The pituitary body is the site of production of the antibody and its decline is secondary to hypopituitarism. This seems unlikely since the operation failed to result in detectable anterior pituitary insufficiency, but was followed by a marked decline in antibody level.

3. The metastatic tumor tissue elaborates the antibody under the influence of an intact hypothalamic pituitary axis. A careful search of the literature has not uncovered any report of tumor tissue producing insulin antibody.

4. The antibody is produced by the usual immune mechanism under the influence of an intact hypothalamic-pituitary axis. The failure of antibody to reappear at significant levels after the renewed insulin stimulus to the immune mechanism strongly suggests such a dependence.

It is possible to discount the nonspecific effects of advancing metastatic disease and cachexia in the initial decline of insulin resistance and its failure to recur in the months following the operation since the patient is thriving and more active than prior to pituitary stalk section. We currently favor the hypothesis that the intact hypothalamic-pituitary axis exerts some influence (possibly a permissive one on an immune mechanism) on the elaboration of insulin-binding globulins. This formulation is being tested in other diabetics who have undergone pituitary stalk section.

SUMMARY

A patient with insulin resistance which subsided after pituitary stalk section is described. The amelioration in diabetes was accompanied by a dramatic decline in the patient's production of an insulin-binding gamma globulin which possessed unusual immunochemical characteristics. A permissive role of the pituitary on elaboration of insulin-binding gamma globulins is suggested.

SUMMARIO IN INTERLINGUA

Le Effecto del Section del Pedunculo Hypophyseari Super le Resistentia a Insulina

Es describite le caso de un patiente con resistentia a insulina que subsideva post le dissection de su pedunculo pituitari. Le melioration del diabete esseva accompaniate de un declino dramatic in le production del insulino-ligante globulina gamma que possedeva inusual characteristicas immunochimic. Es proponite le these que le pituitario exerce un rolo permissive in le elaboration de globulinas gamma capace a ligar insulina.

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Why Human Genetics?

One possible explanation of the wide variation in the organ weights, etc., of highly inbred animals lies in the complexities of the interaction between genes. While inbreeding tends to establish a definite gene pool which is passed on to the offspring, there appears to be no assurance that recombinations might not cause the animals to be heterogeneous because of differences in interactions between the existing genes. In other words, what determines the characters in an animal is not only "the genes with which they are born" but the important variations in the potential interactions of these genes.

Another possible explanation of the widely different patterns of organ weights possessed by inbred animals is somatic mutations. These mutations would have to be very common to produce the effects which have been observed, and for various reasons it seems that this explanation is only possible, but not probable.

Regardless of what interpretation we may accept with respect to the variability in "inbred" strains of animals, a consideration of the facts is highly pertinent in the realm of human genetics because so-called normal human beings exhibit similar enormous variability. Endocrine glands, for example, vary in size and activity over wide ranges—often tenfold or more. Wide variation is the general rule in the realm of composition, enzyme activities, excretion patterns, pharmacological reactions, and nutritional needs, as well as in miscellaneous physiological areas. Furthermore these diversities exist so far as we know within every ethnic group and hence are universal.

In the field of medicine I do not believe that genetic factors are considered as seriously as they should be, and that geneticists are partially to blame. One geneticist, for example, refers to "a patient with a genetic condition." Reference was probably to a patient with hemophilia or some well-established hereditary disease, but the phrase as used suggests that some diseases have no genetic roots. This, in my opinion, is clearly not the case. In the strict sense no patient ever went to see a physician who did not have to greater or lesser degree "a genetic condition." Ample evidence indicates that even susceptibilities to infectious diseases are inherited, though of course the exact modes of inheritance are not known.

By Roger Williams in
Journal of Heredity, Vol. 51,
No. 2, March-April 1960.

The Incidence of Arteriosclerotic Heart Disease in Negro Diabetic Patients

Robert S. Anderson, M.D., Anna Ellington, M.D., and Laurie M. Gunter, Ph.D.,
Nashville

The relative increase in the incidence of arteriosclerotic heart disease has led to a considerable interest in its etiology, pathogenesis and possible predisposing factors. Adequate data on the incidence of this disease in the general population are still insufficient. However, several studies have emphasized a lower incidence in the Negro population. The basis for this conclusion has come mainly from postmortem examinations. There are fewer studies done on living patients.

Phillips and Burch[1] summarized briefly an extensive study of the literature on the racial differences in incidence of cardiovascular disease. They concluded that arteriosclerotic heart disease (including myocardial infarction and angina pectoris) is more frequent in whites than in Negroes, but that the Negroes who die of this disease do so at an earlier age than the whites. The influence of other associated diseases upon the racial difference and the possibility of racial differences in the clinical manifestation of arteriosclerotic heart disease have not been settled.

At present, studies are being done at this institution of the incidence of arteriosclerotic heart disease in the total hospital population, which may help throw further light on the racial incidence of this disease. This report presents one facet of this study and concerns itself with a diabetic population. It is widely accepted that in the latter population, the incidence of arteriosclerotic heart disease is increased over that of the general population. In this regard, it is felt that a study of this disease in a Negro diabetic population may well magnify this difference in incidence in the two races.

CLINICAL MATERIAL

One hundred unselected patients in the diabetes clinic of the George W. Hubbard Hospital of Meharry Medical College were the subjects for this study. The study was conducted from September 1959 to March 1960 on patients who had been observed in this clinic from six months to ten years.

The patients' records were reviewed with particular emphasis on findings related to the cardiovascular system and the following data were obtained and recorded:

1. Age
2. Sex
3. Degree of obesity
4. Average blood pressure
5. Duration of diabetes
6. Type of diabetes therapy
7. Degree of diabetes control
8. Results of teleroentgenograms of the chest and electrocardiographic studies

The records were reviewed for history and evidence of angina pectoris, myocardial infarction and congestive heart failure. When this information could not be documented by a careful analysis of the records, the patients themselves were recalled and this information was obtained when possible.

The degree of obesity was calculated according to the Metropolitan Life Insurance Company tables. The reference for all patients was the average weight for a medium frame.

Blood pressures recorded during the previous months or years were utilized. When the average systolic pressure exceeded 140 mm. Hg and/or the diastolic pressure exceeded 90 mm. Hg, hypertension was considered present. Blood pressures recorded prior to therapy were utilized for patients who were being effectively treated for hypertension. When the patient's record contained an inadequate number of blood pressures, recordings on three consecutive visits were made.

In the majority of cases, teleroentgenograms of the chest were utilized to evaluate cardiac size and the presence or absence of aortic sclerosis. Twelve-lead electrocardiograms were taken with the Sanborn Viso Cardiette electrocardiograph.

The clinical diagnosis of arteriosclerotic heart disease was based on the diagnostic criteria of the New York Heart Association. The patients were placed in

From the Diabetic Clinic of the Department of Medicine, Meharry Medical College, Nashville, Tennessee.

three diagnostic groups. A diagnosis of definite arteriosclerotic heart disease was made when the presence of angina pectoris, myocardial infarction or unequivocal evidence of coronary insufficiency by the electrocardiogram were demonstrated. No patient was chosen on the basis of electrocardiographic evidence alone unless cardiomegaly unexplained by other cardiac lesions and/or aortic sclerosis were present. The second group, termed probable arteriosclerotic heart disease, included those patients with aortic sclerosis and cardiomegaly. The electrocardiograms in this group showed nonspecific changes or were normal. The third group termed potential arteriosclerotic heart disease included those patients who simply had aortic sclerosis without cardiomegaly or other clinical evidence of heart disease that could be definitely attributed to arteriosclerosis. Many of these patients also had nonspecific electrocardiographic changes.

The group studied was composed of twenty-two males and seventy-eight females. The female-to-male ratio in the clinic is approximately three to one. The age range for the males was forty-one to seventy-eight and for the females twenty-three to eighty-eight. The mean age for males was 61.9, and 57.4 for the females with standard deviations of 10.4 years and 12.4 years, respectively. The difference in age for males and females was not statistically significant (using two-tailed probability). These data are summarized in table 1.

The classification of arteriosclerotic heart disease in the present study of 100 diabetic patients is shown in table 2 by sex. Thirty-three patients were found to have definite arteriosclerotic heart disease, twenty-two had probable arteriosclerotic heart disease and twenty-two had potential arteriosclerotic heart disease, nine were classed as "miscellaneous" and fourteen were considered to have no heart disease.

TABLE 1

Arteriosclerotic heart disease in 100 diabetic patients: mean age and age range

	Male	Female
Total number of cases	22	78
Age range (years)	41-78	23-88
Mean age	61.9	57.4

The miscellaneous group included six patients with hypertensive cardiovascular disease. One patient had luetic heart disease with aortic insufficiency and congestive heart failure, and for this reason was not included in the group with potential arteriosclerotic heart disease despite the presence of aortic sclerosis. Another case had isolated cardiomegaly of unknown origin. Finally, one patient with symptoms of angina pectoris, but who was emotionally ill and had no other evidence of arteriosclerotic heart disease was also included in this group.

Cardiovascular Findings

Aortic sclerosis was present in seventy-two of the 100 patients studied (table 3). In the patients with definite arteriosclerotic heart disease, 84.8 per cent had aortic sclerosis. Of those with probable arteriosclerotic heart disease, 95.5 per cent had aortic sclerosis and all of those in the potential arteriosclerotic heart disease group had this lesion. The one patient in the miscellaneous group with luetic heart disease also had aortic sclerosis. It should be noted that the sex incidence of this lesion is approximately equal. The diagnosis of aortic sclerosis was limited to the demonstration by teleroentgenograms of calcium in the aorta.

Table 4 shows the other cardiovascular findings in patients with definite arteriosclerotic heart disease. Angina pectoris occurred in 23 per cent of the total sample and in 66.6 per cent of the patients with definite arteriosclerotic heart disease. There was an equal incidence in the two sexes. Myocardial infarction occurred in five patients, all of whom were females.

Congestive heart failure was present in twenty-five patients. Eighteen had arteriosclerotic heart disease, three had probable arteriosclerotic heart disease, two

TABLE 2

Arteriosclerotic heart disease in 100 diabetic patients (22 males and 78 females)

Sex	Definite ASHD*		Probable ASHD*		Potential ASHD*		Miscellaneous		No heart disease	
	No.	Age range	No.	Age range	No.	Age range	No.	Age range	No.	Age range
Female	27	35-88	14	49-76	19	51-82	6	39-49	12	39-49
Male	6	59-74	8	56-68	3	61-71	3	45-33	2	41-53
Total	33	35-88	22	49-76	22	51-82	9	39-53	14	39-53

*ASHD—Arteriosclerotic heart disease.

had potential arteriosclerotic heart disease and two were in the miscellaneous group. It can be seen in table 4 that a little more than 50 per cent of the patients with definite arteriosclerotic heart disease had congestive heart failure. Again the sexual incidence of this finding is approximately equal.

Of the patients with definite arteriosclerotic heart disease, twenty-two females and five males or 81.8 per cent had either diastolic or systolic hypertension. This complication and its association with congestive heart failure will be discussed later.

Obesity

There was no statistically significant relationship between obesity and arteriosclerotic heart disease. However, it can be seen from table 5 that 78.6 per cent of the patients with no heart disease were less than 20 per cent overweight, while approximately 50 per cent with arteriosclerotic heart disease were overweight.

Hypertension and arteriosclerotic heart disease

Of the 100 diabetic patients, thirty-seven had diastolic hypertension and twenty-two systolic hypertension, making the total incidence of hypertension 59 per cent (table 6). Considering the patients with arteriosclerotic heart disease, 81.8 per cent of those with definite arteriosclerotic heart disease had hypertension (systolic or diastolic). In addition, 45.4 per cent of those with probable, and 63.6 per cent of those with potential, arteriosclerotic heart disease were hypertensive. Of the nine patients with "miscellaneous" heart disease, six had hypertension. Two patients with low-grade systolic hypertension but no other evidence of cardiovascular disease were grouped with the patients considered to have no heart disease. There was no significant difference in the sex incidence of hypertension. The patients with systolic hypertension were older than those with diastolic hypertension. This finding was significant at the .01 level using the t test. The meaning of this finding, however, has not been explored.

Sixteen of the twenty-seven patients (59 per cent) with hypertension and definite arteriosclerotic heart dis-

TABLE 3
Incidence of aortic sclerosis in 100 diabetic patients by sex

	Definite ASHD* 33		Probable ASHD* 22		Potential ASHD* 22		Miscellaneous 9		No heart disease 14		Total 100	
Aortic sclerosis	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Female	23	85.1	13	92.8	19	100.0	—	—	—	—	55	70.5
Male	5	83.3	8	100.0	3	100.0	*1†	33.3	—	—	17	77.2
Total	28	84.8	21	95.5	22	100.0	1	33.3	—	—	72	72.0

*ASHD—Arteriosclerotic heart disease.
†Luetic heart disease.

TABLE 4
Cardiovascular findings in thirty-three patients with definite arteriosclerotic heart disease

	Aortic sclerosis	Angina pectoris	Myocardial infarction	Congestive heart failure	Hypertension (systolic & diastolic)	Total
Total number	28	22	5	18	27	33
Per cent female	85.1	66.6	18.5	55.5	81.4	81.8
Per cent male	83.3	66.6	0	50.0	83.3	18.2
Per cent of total	84.8	66.6	15.1	54.5	81.8	100.0

TABLE 5
Degree of obesity in 100 diabetic patients

Degree of obesity	Definite ASHD* 33	Probable ASHD* 22	Potential ASHD* 22	No heart disease 14	Miscellaneous 9	Total 100
			Per cent			
20 per cent or more	48.5	59.1	45.4	21.4	55.6	47.0
Less than 20 per cent	51.5	40.9	54.6	78.6	44.4	53.0

*ASHD—Arteriosclerotic heart disease.

TABLE 6
Arteriosclerotic heart disease and hypertension in 100 diabetics

	Definite ASHD*	Probable ASHD*	Potential ASHD*	Miscellaneous	No heart disease	Total
Hypertension	33	22	22	9	14	100
Present (per cent)	81.8	45.4	63.6	66.6	14.3	59
Absent (per cent)	18.2	54.6	36.4	33.3	85.7	41

*ASHD—Arteriosclerotic heart disease.

ease also had congestive heart failure; eleven had diastolic and five had systolic hypertension. In the total sample only 20 per cent of the patients with congestive heart failure did not have hypertension, or stated another way, 80 per cent of the patients with congestive heart failure had hypertension.

DISCUSSION

The male-to-female ratio in this study is similar to those in many reports involving predominantly white diabetic populations.[2-5]

In view of the wide range in incidence of aortic sclerosis reported in diabetic patients (from 24 to 75 per cent)[5-7] there seems to be a need for better controlled studies of this lesion. Certainly the present study suggests that the incidence of this lesion is as high or higher in Negro than in white diabetic patients.

The cardiovascular findings in patients with definite arteriosclerotic heart disease as compared with the findings of other investigators show that the incidence of angina pectoris is higher in this group of patients than in other recently reported clinical studies. Edeiken[7] who studied 100 diabetic patients found angina pectoris in eight and indefinite chest pain in five others. All belonged to the older age group and of the eight with angina pectoris there were four men and four women. The five cases with indefinite chest pain were women. Angina pectoris was found in Liebow's study[6] in 10.2 per cent of the cases. Bryfogle and Bradley[8] reported that angina pectoris was present in 12.3 per cent of their patients over the age of thirty-nine. All of these studies involved predominantly white patients.

The absence of myocardial infarction in the male patients in this study is unexplained, although the male sample is so small other factors must be considered. Thomas[9] studied ninety Negro patients with myocardial infarction admitted to the George W. Hubbard Hospital during a twenty-year period ending January 1960. There were fourteen patients in this series with diabetes, eleven female and only three male. In the total series there were fifty male and forty female patients. Both the present study and that of Thomas[9] involving Negro patients suggest a higher incidence of myocardial infarction in the female when diabetes is present. This is an interesting though unexplained finding. The over-all incidence of 5 per cent is below those reported in the recent literature.[6,8]

The prevalence of hypertension in the present series is generally higher than in other clinical studies.[6,7,8] While diastolic hypertension was distinguished from systolic hypertension, a thorough analysis has not been made in terms of these two types of hypertension. The data did not confirm the higher incidence of hypertension in females as reported in the studies referred to above. Finally, we cannot escape the suggestion that the high incidence of hypertension in patients with definite arteriosclerotic heart disease relates itself to the prevalence of this disease as well as to the high incidence of congestive heart failure in this group.

SUMMARY

One hundred unselected Negro patients from the diabetes clinic of George W. Hubbard Hospital of Meharry Medical College were studied for the prevalence of arteriosclerotic heart disease. Thirty-three patients were found to have evidence of definite arteriosclerotic heart disease, twenty-two probable arteriosclerotic heart disease and twenty-two potential arteriosclerotic heart disease. Considering all categories of arteriosclerotic heart disease, the prevalence in this group would be 77 per cent. Compared to other clinical studies, these findings do not support a lower incidence of arteriosclerotic heart disease in a Negro indigent diabetic population as compared with a predominantly white diabetic population.

The incidence of aortic sclerosis, angina pectoris and myocardial infarction has been recorded and compared with studies involving predominantly white patients. The relationship of obesity to heart disease has been noted. Finally, the incidence and the interrelationships of hypertension and congestive heart failure to arteriosclerotic heart disease have been studied and discussed.

SUMMARIO IN INTERLINGUA

Le Incidentia de Arteriosclerotic Morbo Cardiac in Patientes Diabetic de Racia Negre

Cento non-seligite patientes negre ab le clinica dia-

betic del Hospital George W. Hubbard al Collegio Medical Meharry esseva studiate con respecto al prevalentia de arteriosclerotic morbo cardiac. Esseva constatate que trenta-tres patientes exhibiva manifestationes definite de arteriosclerotic morbo cardiac; vinti-duo habeva probabilemente arteriosclerotic morbo cardiac; e vinti-duo alteres habeva le potentialitate de arteriosclerotic morbo cardiac. Si omne le mentionate categorias es combinate, le prevalentia de arteriosclerotic morbo cardiac in iste gruppo amonta a 77 pro cento. Comparate con altere studios clinic, iste constationes non supporta le these de un plus basse incidentia de arteriosclerotic morbo cardiac in un population diabetic de negros indigente que in un population diabetic de racia predominantemente blanc.

Le incidentia de sclerosis aortic, de angina de pectore, e de infarcimento myocardial esseva registrate e comparate con studios in patientes de racia predominantemente blanc. Le relation inter obesitate e morbo cardiac es notate. Finalmente, es studiate e discutite le relation inter, de un latere, hypertension e disfallimento cardiac congestive e, del altere latere, arteriosclerotic morbo cardiac.

ACKNOWLEDGMENT

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We are indebted to Dr. John Thomas for evaluation of the electrocardiograms and to Dr. G. J. Tarleton for the interpretation of the chest X rays.

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Hypoglycemia and Heart Disease

Because of the frequency of coronary sclerosis in patients with diabetes mellitus, E. S. Egeli and R. Berkmen (*Am. Heart J.* 59:527, 1960) decided to study hypoglycemic reactions in a group of nondiabetic patients who had coronary arterial disease. They selected sixteen patients who had angina pectoris and twenty-two patients who had had myocardial infarctions in the past, and these thirty-eight patients were then divided into four groups. After the administration of the test substance, blood was drawn at intervals of fifteen minutes for a total of two hours and determinations were made of the sodium, potassium, calcium and glucose content. Electrocardiograms were taken at the same intervals.

The first group of fourteen subjects was given an insulin tolerance test (0.2 units per kg. of body weight). The second group was given the same quantity of insulin but was given potassium chloride either orally in a dose of 5 gm. before administration of insulin or by intravenous injection in the amount of 2 gm. after the effects of insulin had become apparent. A third group of eight patients was given the same amount of insulin, but in addition was given phentolamine thirty to forty-five minutes later. The fourth group of patients was given an injection of epinephrine alone.

The results confirmed the observations of other groups (A. Keys, *Am. J. Physiol.* 123:608, 1938). The patients developed clinical and biochemical manifestations of hypoglycemia and their electrocardiograms showed changes, primarily in the ST segments and T-waves. Measurements of potassium, sodium and calcium disclosed a marked hypokalemia, a mild hypernatremia, and no significant change in the calcium. In the second group, administration of potassium chloride afforded a remarkable degree of protection against the effects of hypoglycemia. The electrocardiographic changes were prevented and there was some inhibition of the hypoglycemic effect of insulin. In three instances, hypoglycemia was allowed to develop before potassium chloride was given by injection, whereupon the electrocardiographic changes were reversed promptly. The third group developed changes similar to those of the first and phentolamine gave no apparent protection. The fourth group developed arrhythmias and changes in the ST segment and T-waves similar to those seen in group one.

From *Nutrition Reviews*, Vol. 18, No. 9, p. 263, September 1960.

A Clinical Assessment of Fetal Calf Insulin

J. M. Salter, Ph.D., O. V. Sirek, M.D., Ph.D., M. M. Abbott, M.D., and B. S. Leibel, M.D., Toronto

Early in their original work with insulin, Drs. Banting and Best were confronted with the problem of discovering a source of the hormone to meet the inevitable demands of the diabetic population throughout the world. At that time, the extraction of insulin seemed to depend upon the prior elimination of the external secretory portion of the pancreas. With this in mind Banting and Best, while working alone, explored the possibilities that fetal pancreas, before the development of its exocrine function, might provide a useful source of the hormone. Although fetal calf insulin was prepared in 1921 and used with dramatic success in diabetic dogs, it was never tested clinically, presumably because of an abundance of insulin soon made available from adult cattle by alternative technics.[1] Now, almost forty years later, a series of clinical trials of this substance have been initiated in order to fill this gap in the history of insulin.

METHODS

Banting's and Best's original notes were consulted and the insulin was prepared according to the procedure they used in 1921. Banting and Best used the pancreas immediately after they had removed it from the fetuses. Since we wished to prepare relatively large amounts of fetal insulin at regular intervals, it was necessary to collect and freeze the glands whenever they became available.

Two hundred grams of frozen fetal pancreas were cut into thin shavings and extracted for four hours in 200 ml. of acid alcohol (95 per cent ethanol, 0.6 concentrated HCl). The extraction was performed with continuous agitation at a temperature of 5 degrees C. The mixture was then filtered (later lots were centrifuged) and the filtrate was saved. The tissue debris was re-extracted for four hours with another 200 ml. of acid alcohol. The filtrates from the extractions were combined and evaporated nearly to dryness in a small vacuum still. The residue was dissolved in 50 ml. of saline. The insulin content was assayed by the mouse convulsion technic and was found to be 24 units per ml. Berkefeld filtration provided sterility.* The insulin solution administered to the children (patients R.K. and I.D.) was diluted to 12 units per ml. while that administered to the adults was diluted to 20 units per ml.

Prior to its clinical use, two volunteers received small subcutaneous or intracutaneous injections of the fetal insulin extract on four separate occasions. It produced no detectable irritation or side effects.

RESULTS

At the Hospital for Sick Children,† Toronto, three children with diabetes of recent onset, while in the hospital for stabilization, volunteered for this experiment. They were treated with diet and were receiving 12 to 25 units of Lente and Crystalline Zinc Insulin. The material to be tested was injected in the morning, twenty to twenty-four hours after the last dose of insulin. The patients were fasted overnight and their breakfast consisted only of eight ounces of unsweetened orange juice, given approximately two hours before the experiment.

Fetal calf insulin, Lot CHB-2, was injected subcutaneously in a boy, R.K., and in a girl, I.D. Both were seven years old. Figure 1 shows the effect of this material upon blood sugar concentration and the precipitous fall is quite evident when compared to the blood sugar curve obtained on a thirteen-year-old diabetic boy who received 0.5 cc. of saline under the same experimental conditions. The material was well tolerated and there were no side reactions of any kind.

It was possible to compare fetal calf insulin with the

From the Banting and Best Department of Medical Research, the Department of Physiology, University of Toronto, and Baycrest Hospital, Toronto, Canada.

*Recent experiments carried out in this laboratory indicated that the original procedure also extracts a substance (possibly an enzyme) that destroys insulin over a three- to four-month period. This slow inactivation was not evident during the early work because preparations were used promptly. Heating the insulin preparation rapidly to 70° C. for three minutes and then cooling, prevents subsequent deterioration and allowed more prolonged storage of the fetal insulin. This heating procedure was used in the preparation of the later lots of material.

†The facilities of the Hospital for Sick Children were provided by Professor A. L. Chute.

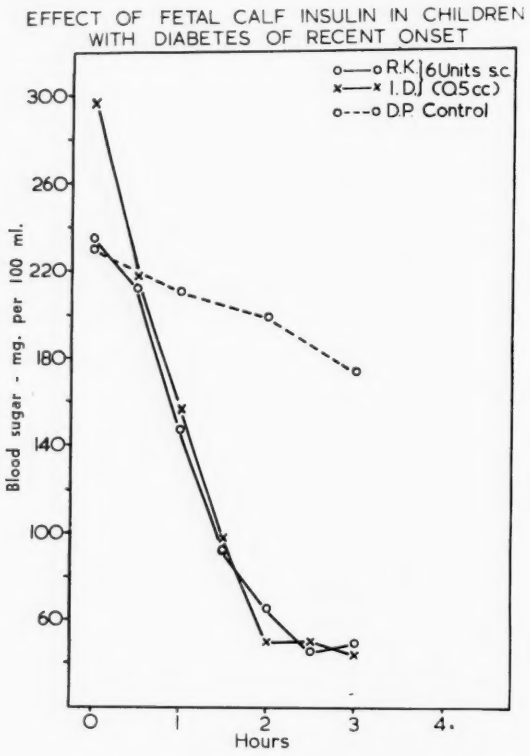

FIGURE 1

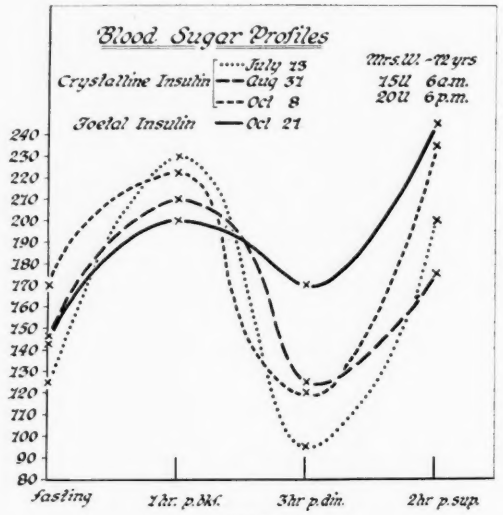

FIGURE 2

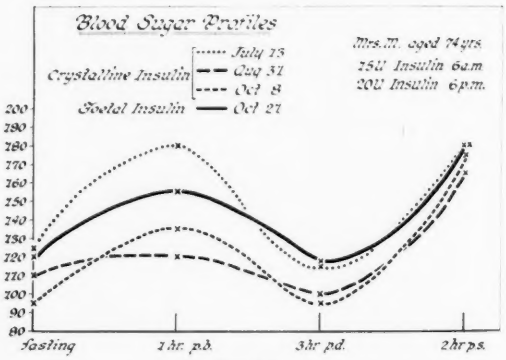

FIGURE 3

action of Crystalline Zinc Insulin at the Baycrest Hospital* for the chronically ill in Toronto. At this institution, the patients are in permanent residence and physical activity, emotional environment and dietary intake are constant within practical limits. The subjects selected for study were changed from their usual depot insulin to divided doses of Crystalline Zinc Insulin. A baseline was established by determining their blood sugar profiles which were repeated at three widely spaced intervals. These were followed in each case by a similar survey after the patient had been receiving the same doses of fetal insulin for a period of two weeks. The results of these investigations are displayed on the succeeding graphs. During the course of the latter therapy no local irritation at the sites of injection was seen, nor were there any remote toxic effects from the fetal calf insulin.

Mrs. E.W., aged seventy-seven years, was discovered to be suffering from diabetes seven years ago. Twenty years ago she had a myocardial infarction and developed congestive cardiac failure which has been controlled satisfactorily. Three years ago she fractured her left hip which was pinned successfully. The control of her diabetes mellitus has been uneventful.

Mrs. J.M., aged seventy-five years, developed diabetes six years ago. At first she was controlled by diet alone, but subsequently also required insulin. In 1957 she was changed to tolbutamide therapy. Following a good response initially, she relapsed in six months and was then placed on insulin therapy. Nine years ago she had a posterior myocardial infarction and in recent months has been under therapy for essential hypertension and cerebrovascular disease.

*The clinical investigations of diabetes at Baycrest Hospital are supported by the S. Lunenfeld Charitable Foundation.

CONCLUSIONS

Fetal calf insulin, prepared by the original technic of extraction and purification used by Banting and Best in 1921, exerts the same effect in diabetic patients as does modern Crystalline Zinc Insulin. The fetal calf insulin produced no local reactions and presumably would have provided a useful source of hypoglycemic material for the initial treatment of cases of diabetes mellitus in Toronto in 1922 if a more readily available source (adult beef pancreas) had not proved satisfactory.

SUMMARIO IN INTERLINGUA

Evalutation Clinic de Insulina ab Vitellos Fetal

Insulina de vitello fetal, preparate secundo le technica original de extraction e purification que esseva usate per Banting e Best in 1921, exerce le mesme effecto in diabeticos como le moderne insulina a zinc crystallin. Le insulina de vitello fetal produceva nulle reactiones local e, sin dubita, haberea representate un satisfacente materia hypoglycemic in le tractamento initial de casos de diabete mellite in Toronto in 1922, si le plus prestemente accessibile fonte de pancreas bovin adulte non se habeva provate adequate.

ACKNOWLEDGMENT

This work was supported in part by the Samuel Lunenfeld Charitable Foundation.

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Because a considerable proportion of overweights who reduce fail to maintain their lower weight, those who succeed in keeping their weight down for a time constitute a select group. However, this can be only a partial explanation of the favorable findings for those who reduced their weight. Evidence of specific benefits from weight reduction is available from other sources. One striking illustration was the decrease in mortality—particularly from cardiovascular disease and diabetes—in many parts of Europe during World War II, when food supplies were drastically restricted, and the general reversal of this trend when food became more abundant. The great improvement in the mortality of American women reflects, in part, the benefits of weight control, as shown by the decrease in average weight of women. Men, whose average weight increased in the past generation, experienced a much smaller reduction in mortality.

On the clinical side, weight reduction is virtually routine in the treatment of heart disease and hypertension. Reduction of excess weight decreases the strain on the heart, and thus tends to lower blood pressure. The Build and Blood Pressure Study provides abundant evidence of the rise in blood pressure with increase in degree of overweight. A recent study of elderly obese patients in a nursing home—most of them hypertensives—showed that almost all those who reduced their weight experienced a marked decrease in blood pressure. Studies of middle-aged overweight diabetics indicated that weight reduction improved their glucose tolerance, and in those who reduced to average weight, normal tolerance was restored in most cases.

Overweight is a more significant factor in longevity now than ever before because of its association with the cardiovascular diseases, which now account for the majority of all deaths. The situation is particularly serious among males. Accordingly, whatever measures are effective in reducing the death toll from these diseases are likely to make the greatest contributions to further improvement in health and longevity.

The reduction of weight and its maintenance at optimum levels depend upon the relation of caloric intake to caloric utilization. Overweights can reduce only if they consume less than their body needs. A reasonable and persistent program of diet and exercise, under the continuous guidance of a physician, and the avoidance of fad diets will accomplish the desired result.

From *Statistical Bulletin,*
Vol. 41, pp. 2-3, April 1960.

Pancreatic Function in Alloxan "Subdiabetic" Rats Long-term Treated with Tolbutamide

Jann W. Weber, M.D., Jean-Pierre Colombo, M.D., Richard I. Goldberg, B.A., Stanley Saperstein, B.A., Martin L. Shulkind, B.A., Doris Kanameishi, M.T., and Piero P. Foa, M.D., Chicago

It is generally believed that one of the reasons for the blood-sugar-lowering effect of the sulfonylureas is the release of insulin from the pancreas. This release has been documented convincingly in acute experiments.[1] However, one cannot say if this is due primarily to a discharge of preformed insulin, as the degranulation of the beta cells would suggest, or if secretion of newly synthesized insulin also occurs. The variety of animal species and strains, of ages and physiologic conditions, of drugs, dosage schedules and lengths of the experiments makes the effects of prolonged administration even harder to interpret. The problem is complicated further by the well-known difficulties which arise in assessing function from morphologic evidence, and in comparing morphologic data obtained at different times, with different technics in a different, or even in the same, laboratory.[2-5]

In an attempt to render their interpretation somewhat easier, pertinent data from the literature[5-31] have been tabulated and the period of 100 days, or about 10 per cent of the life span of a rat, has been selected as the arbitrary division between "short" and "long" experiments (table 1). From this table, one gains the impression that, in most "short" experiments in the normal animal,[6-9,14-18] the drugs either had little or no effect on pancreatic function and morphology, or resulted in beta-cell degranulation with some impairment of the animal's tolerance for glucose. On the other hand, in most "long" experiments,[10-14] stimulation of insular function, such as islet hypertrophy, beta-cell multiplication and increased glucose tolerance have been obtained. In the diabetic animal, the effect of sulfonylurea administration appears to have been determined more by the severity of the disease than by the duration of treatment. With the exception of one report,[24] when diabetes was mild or moderate, improvement, "remissions," and "cures" have been described, whereas in severe cases no significant effects have been obtained.[19-23,25-31] In addition to duration of treatment and severity of disease, some of the results may have been influenced by other factors. In some cases, the tendency of rodents to spontaneous recovery from alloxan diabetes[32] may have played a part; in other cases, changes in liver function[33] or glycogen content[1] may have influenced carbohydrate tolerance; in still other cases the response of the drug may have depended upon the pancreatic reserve of the animal. The extent of this reserve[34] may determine if continued stimulation of the beta cell will result in hypertrophy and increased insulin production or lead to exhaustion and functional insufficiency, a situation comparable to that observed after repeated doses of growth hormone, prolactin, cortisone, glucagon or glucose.[35-37] Beta cell exhaustion, leading to further impairment of the basic endocrinologic defect, is a serious matter and the possibility that it might happen in diabetic patients should be considered, even though countless diabetics have received oral therapy for several years and, in the relatively few cases of secondary failure, no significant increase in insulin requirement seems to have occurred.[38-40] For these reasons, the problem was reinvestigated in rats whose pancreatic reserve had been decreased by a subdiabetic dose of alloxan. It was hoped that, in these animals, any significant changes in pancreatic function could be detected either by an improvement in glucose tolerance or by the appearance of frank diabetes.

MATERIALS AND METHODS

About 200 Sprague-Dawley (Abrams) rats, weighing about 120 gm., were fasted overnight, and given a single intraperitoneal injection of alloxan monohydrate

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From the Department of Physiology and Pharmacology of The Chicago Medical School, Chicago 12, Illinois.

(150 mg./kg. in a freshly prepared 5 per cent solution). Eight to ten days after the injection, the surviving rats were again fasted overnight and tested for urinary glucose. Rats with glycosuria were used for other purposes; those with negative urine were given an intraperitoneal glucose tolerance test (1 mg./kg. in a 10 per cent solution). Thirty-one aglycosuric rats, now weighing about 150 gm., having normal fasting blood sugar, but impaired glucose tolerance, remained. These were divided into two groups by a person who had no knowledge of each animal's tolerance curve. One group received a commercial diet and served as control; the second group received the same diet to which 2 per cent tolbutamide had been added. Occasional measurements of food consumption revealed that the daily drug intake was about 100 mg./rat, or 650 mg./kg. at the beginning of the experiment, and about 200 mg./kg./rat, or 500-600 mg./kg. at the end of the experiment, when the weight of the animals had increased to about 300-400 gm. Intraperitoneal glucose tolerance tests were repeated at various intervals, after an overnight fast and after discontinuing the drug for three days—one day for complete absorption of tolbutamide from the gastrointestinal tract, one day for its disappearance from plasma and tissues[41] and one day for the abatement of any acute pharmacologic effects of the last dose. Among these, insulin discharge, hypoglycemia and reactive secretion of blood sugar raising hormones[42] may cause changes in glucose tolerance. Duplicate blood samples were obtained from the cut surface of the tail and glucose was determined according to Nelson.[43] From the blood glucose values, two indices were calculated: an "index of comparison," calculated as follows:

$$I_{Co} = \frac{\text{1-hr. exper. blood glucose}}{\text{1-hr. control blood glucose}} \cdot \frac{\text{x-hr. exper. blood glucose}}{\text{x-hr. control blood glucose}}$$

and an "index of change," calculated as follows:

$$I_{Ch} = \frac{\text{1-hr. bld. gluc. at 12-15 mos.}}{\text{1-hr. blood glucose at start}} \cdot \frac{\text{x-hr. bld. gluc. at 12-15 mos.}}{\text{x-hr. blood glucose at start}}$$

where $x = 2$, 3 or 4. I_{Co} compares experimental to control animals at a given time; I_{Ch} compares those rats of a given group who survived at the end of the experiment to the same rats at the beginning of the experiment. An index of comparison of 1.0 is obtained when the values for the two groups are identical. An index of comparison greater or smaller than 1.0 indicates that the experimental animals are "more diabetic" or "less diabetic" than the controls, respectively. On the other hand, an index of change smaller than 1.0 indicates that the animals of a given group have improved and an index of change greater than 1.0 that they have deteriorated during the period of observation. The statistical significance of the results was calculated according to Fisher.[44] At the end of the experiment the animals were sacrificed with a blow on the head, the pancreas immediately removed, fixed in Bouin's solution and stained according to Gomori.[45] For better comparison and to minimize irregularities in staining and other procedures, all sections were stained simultaneously, photographed in color on the same day, using the same illumination and film batch, and all photographs were processed together.

RESULTS

Figure 1 shows the average glucose tolerance curve of control and experimental subdiabetic rats. The fasting blood glucose varied between 63 and 118 (average 85) mg. per 100 ml. in the experimental animals, and between 70 and 122 (average 95) mg. per 100 ml. in the controls. Statistical calculation demonstrated no significant differences between the initial blood sugar concentrations and between all other corresponding values in the glucose tolerance curves in the two groups. Figure 2 shows the deterioration of glucose tolerance in the control group, occurring after twelve to fifteen months of observation. This deterioration was noted in eight of the ten control rats surviving at the end of the experiment; two rats were unchanged. In figure 2 only the values at two, three and four hours in the

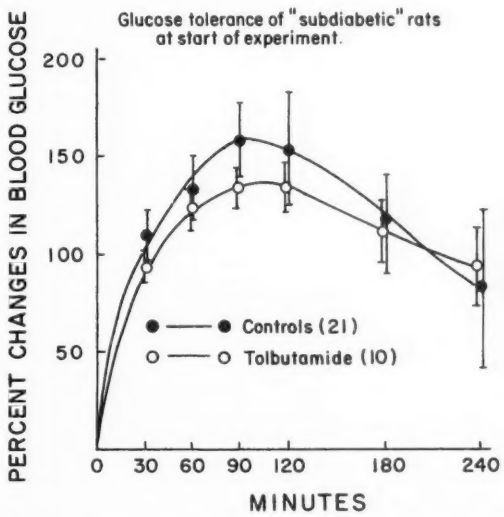

FIG. 1. Average glucose tolerance curves of alloxan "subdiabetic" rats at the beginning of the experiment. ± S.E.

TABLE 1

Effect of repeated doses of oral hypoglycemic drugs on pancreatic morphology and function. Data from the literature. h = hours; d = days; w = weeks; m = months; y = years; C = carbutamide; T = tolbutamide; Ch = chlorpropamide; 2254RP, or IPTD = p-aminobenzene-sulfamido-isopropyl-thiodiazol; 2259RP = p-amino-benzene-sulfamido-tertiobutyl-thiodiazol; S = "short" experiments; L = "long" experiments; g.t. = glucose tolerance; FBS = fasting blood sugar.

Animal	Weight or age	Drug	Dose		Duration of experiment		Effects	Ref.
			Normal animals					
Rat	50-55 gm.	C	1,000	mg./kg.	5 w	S	Increased islet tissue.	6
"	150-200 gm.	C	500	"	9-13 w	S	Gradual increase in FBS; B cell degranulation	7
"	130-160 gm.	C or T	750	"	4-45 d	S	No changes in islet morphology.	8
		IPTD	750-1,000	"	2-45 d	S		
"	—	T	250-500	"	5-80 d	S	B cell degranulation; no change in g.t.	9
"	55-65 gm.	T	100	"	25-192 d	L	Possible inhibition of B cell multiplication.	10
"	130-250 gm.	C	25-500	"	12 h-7 m	L	Islet hypertrophy and hyperplasia; B cell degranulation and enlargement of nuclei.	11
"	175-450 gm.	C	250	"	4-100 d	L	Increased islet tissue; increase and degranulation of B cells.	12
"	200-250 gm.	C	500	"	6 m	L	Islet hypertrophy; B cell degranulation and vacuolization; normal g.t.	13
"	—	T	250-2,000	"	9 m	L	B cell degranulation; increased g.t.	14
Mouse	24-28 gm.	C	500	"	2 m	S	Slight islet hypertrophy and neoformation.	15
"	—	C	500	"	1-3 w	S	Potentiation of alloxan effect.	16
Rabbit	1.8-2.2 kg.	T	1,000	"	3-60 d	S	Decreased g.t.	17
"	—	T	500-1,000	"	4-8 w	S	B cell degranulation; no change in g.t.	9
Dog	—	T	100	"	9 m	L	Increased g.t.	14
"	—	T	100	"	4-6 w	S	Decreased g.t.	13
Calf	70 kg.	T	100	"	4 w	S	Slight decrease in pancreatic insulin content.	18

twelve- to fifteen-month curve and the value at four hours in the nine-month curve are significantly different from the corresponding values at the beginning of the experiment. The deterioration of the control rats is revealed also by the index of change which, after twelve to fifteen months of treatment, was 1.44 at two hours, 2.25 at three hours, and 2.34 at four hours. Figure 3 shows that the glucose tolerance of the experimental rats deteriorated during the first six months, but returned to the original, or possibly better, values after twelve to fifteen months of continuous treatment. At this time the index of change was 0.71 at two hours, 0.67 at three hours and 0.68 at four hours. Of the seven experimental rats remaining at the end of the experiments, four had improved and three were unchanged. In figure 3 only the values at two to three and five to six months are significantly different from the corresponding values at the beginning of the experiment. Figures 4 and 5 show that, during the first six months, tolbutamide feeding does not prevent and may tend to aggravate the diabetic state of the animals. Figure 6 shows that twelve to fifteen months after the beginning

TABLE 1 (CONTINUED)

Effect of repeated doses of oral hypoglycemic drugs on pancreatic morphology and function. Data from the literature. h = hours; d = days; w = weeks; m = months; y = years; C = carbutamide; T = tolbutamide; Ch = chlorpropamide; 2254RP, or IPTD = p-aminobenzene-sulfamido-isopropyl-thiodiazol; 2259RP = p-amino-benzene-sulfamido-tertiobutyl-thiodiazol; S = "short" experiments; L = "long" experiments; g.t. = glucose tolerance; FBS = fasting blood sugar.

Animal	Weight or age	Drug	Dose	Duration of experiment	Effects	Ref.
			Diabetic animals			
Alloxan rat	150-200 gm.	C	500 mg./kg.	9-13 w	B cell degranulation; no effect on diabetes.	7
"	—	2254RP	400 "	7 d	B cell degranulation; no effect on diabetes.	19
Alloxan rabbit	1.3-3.3 kg.	T; 2254RP	100 "	1-18 w	Remission or "cure" of diabetes.	20
"	1.8-2.5 kg.	T	1,000 "	5 d	Improvement of diabetes in mild or moderate cases.	21
"	—	2254RP	500-1,000 "	7 d	Improvement or "cure" of diabetes.	19-22
Alloxan dog	—	2259RP	2,000-2,500 "	36 h-21 d	Islet neoformation; "cure" of diabetes.	22-23
Alloxan subdiabetic rat	3-4 mg.	T	200-500 "	12 m	Decreased g.t.	24
Partially depancreatized rat	180-220 gm.	T	250 "	91 d	Reduced g.t.; "reactive" islet regeneration.	25
Metahypophyseal diabetic dog	10-17 kg.	T	50-100 "	4 w	Suppression of the diabetogenic effect of STH.	26
Obese-hyperglycemic mouse	55-64 gm.	C	300 "	2 m	No effect on diabetes.	15
Man	9 y	C	0.5 g/d	3-4 m	Increased g.t.	27
"	11-35 y	T	0.5 g.; bid	2-22 m	Normalization of g.t.	28
"	Adult	C	1 g/d	3 m	Increased g.t.	29
"	" (?)	C or T Ch.	1 g/d	38-96 h	Increased g.t.	5
"	"	C or T	—	1-21 m	Possible islet neoformation.	30
"	"	T	2 g/d	up to 6 m	No morphologic changes.	31

of the experiment this trend is reversed, resulting in a marked difference in glucose tolerance between control and tolbutamide-fed rats. At this time the initial blood glucose was 75 to 114 (average 84) mg. per 100 ml. in the controls and 80 to 114 (average 91) mg. per 100 ml. in the experimental animals. Statistical analysis shows that these two curves, after starting at similar levels, differ significantly at all succeeding points. (P smaller than 0.02 or better.) The index of comparison, which was 0.93 at two hours and 1.0 at three and at four hours when the experiment was started, had decreased to 0.34 at two hours, 0.22 at three hours and 0.17 at four hours after twelve to fifteen months of treatment, confirming the fact that, at the end of the experiment, the tolbutamide-fed rats were significantly "less diabetic" than the controls. Figure 7 shows a typical example of the degranulated islets of Langerhans observed in control rats. Figure 8 shows a typical pancreatic islet of a tolbutamide-fed rat with abundant beta granules. No differences in the number and appearance of the alpha cells, or in the number and size of the pancreatic islets were noted on careful examination of several sections from all rats of each group, although no serial sections were made.

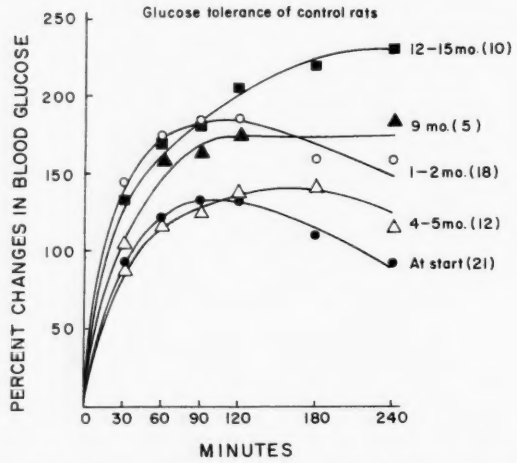

FIG. 2. Average glucose tolerance curves of alloxan "subdiabetic" rats after receiving a control diet for various periods of time.

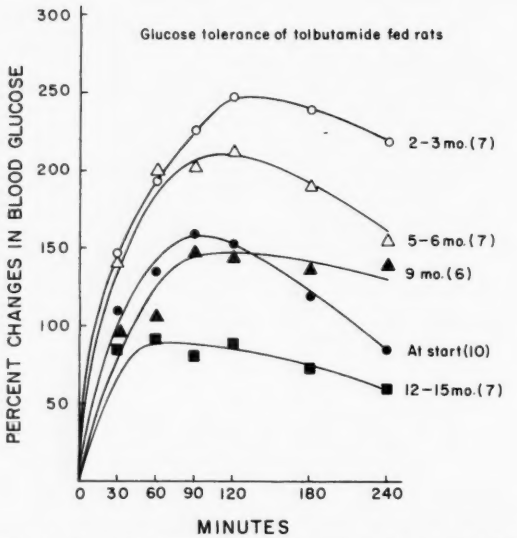

FIG. 3. Average glucose tolerance curves of alloxan "subdiabetic" rats after receiving a tolbutamide containing diet for various periods of time.

DISCUSSION

The deterioration of glucose tolerance in untreated animals confirms the tendency to spontaneous progression of alloxan diabetes observed by others,[24] and suggests that the marked degranulation of the beta cells observed in these rats at the end of the experiment was a lasting sign of the cellular damage caused by alloxan.

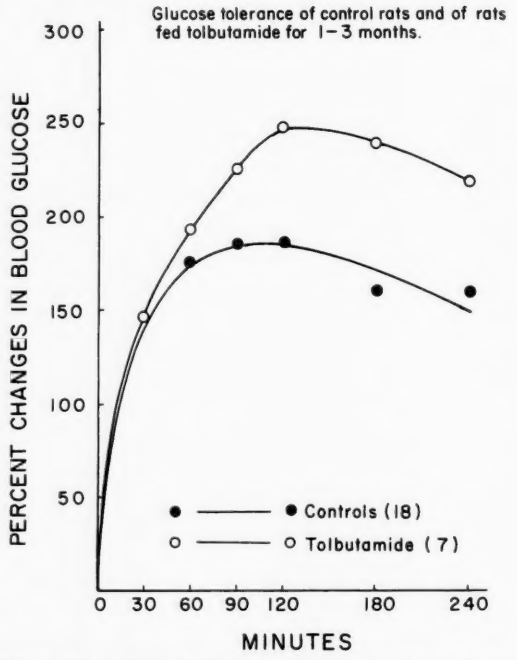
Glucose tolerance of control rats and of rats fed tolbutamide for 1–3 months.
PERCENT CHANGES IN BLOOD GLUCOSE
MINUTES
Controls (18)
Tolbutamide (7)

FIG. 4. Comparison of the glucose tolerance curves of control and tolbutamide-fed "subdiabetic" rats one to three months after the beginning of the experiment. Data from figures 2 and 3.

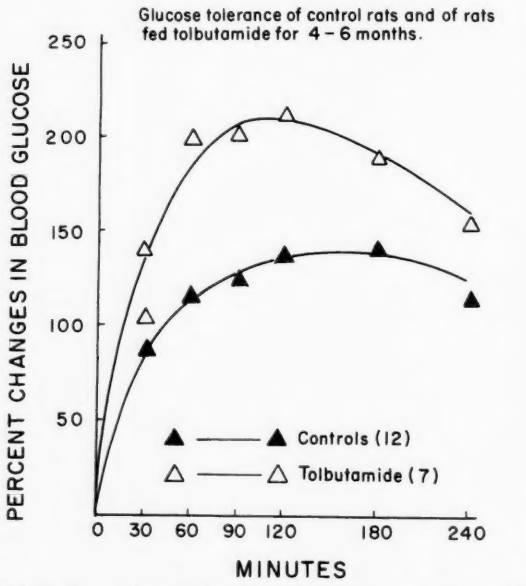
Glucose tolerance of control rats and of rats fed tolbutamide for 4–6 months.
PERCENT CHANGES IN BLOOD GLUCOSE
MINUTES
Controls (12)
Tolbutamide (7)

FIG. 5. Comparison of the glucose tolerance curves of control and tolbutamide-fed alloxan "subdiabetic" rats four to six months after the beginning of the experiment. Data from figures 2 and 3.

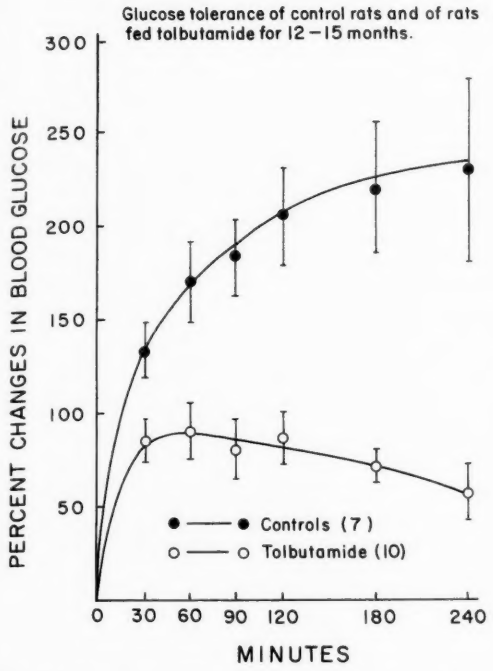

FIG. 6. Comparison of the glucose tolerance curves of control and tolbutamide-fed alloxan "subdiabetic" rats twelve to fifteen months after the beginning of the experiment. ± S.E.

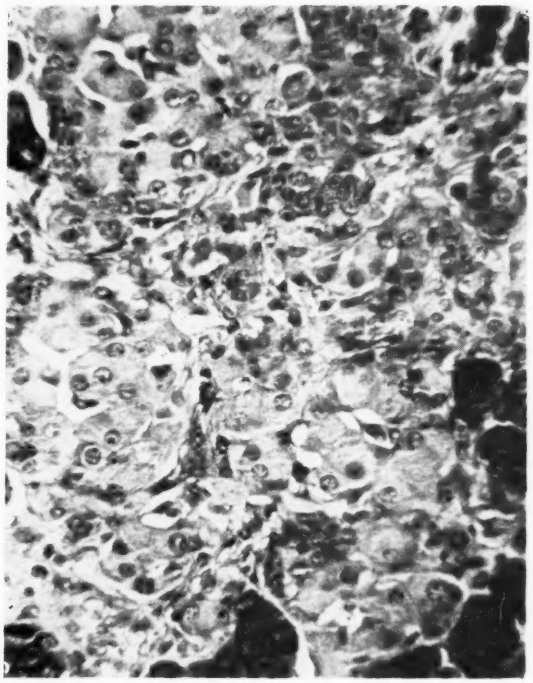

FIG. 7. Islet of Langerhans from a control alloxan "subdiabetic" rat showing characteristic degranulation of the beta cells. A few alpha cells can be seen in the upper left portion of the figure. Gomori stain X 400.

In no case did spontaneous amelioration occur. On the other hand, in the treated animals, after an initial deterioration lasting about six months, there was an increased tolerance for glucose, coincident with the appearance of abundant beta granules, suggesting restoration of islet function. These results are in partial agreement with those obtained by Lazarow and Treibergs[24] under similar, although not identical, circumstances. These investigators reported that tolbutamide feeding produced an impairment in the glucose tolerance during the first six months of treatment, but did not report any figures for the twelve- to fifteen-month period. It should be pointed out that the effects of chronic tolbutamide feeding were not very striking. Neither was the initial impairment of glucose tolerance sufficient to change subdiabetic rats into frankly diabetic ones, nor did the subsequent improvement result in a "cure."

In conclusion, the results described in this paper suggest that prolonged treatment of alloxan subdiabetic rats with tolbutamide does not cause further deterioration of insular function and may even result in some improvement.

SUMMARY

Alloxan-treated aglycosuric rats having normal fasting blood glucose, but abnormal glucose tolerance, were fed a control diet or a similar diet containing 2 per cent tolbutamide. After twelve to fifteen months of treatment, the glucose tolerance of the tolbutamide fed rats was significantly better than that of the controls. At this time, the islets of the control animals were markedly degranulated; those of the tolbutamide fed animals appeared normal. It is concluded that chronic tolbutamide feeding does not cause, and may prevent, further deterioration of pancreatic function in rats with decreased pancreatic reserve.

SUMMARIO IN INTERLINGUA

Le Function Pancreatic in Rattos "Subdiabetic" per Alloxano, Tractate a Longe Vista con Tolbutamida

Rattos, le quales—post tractamento con alloxano—esseva aglycosuric e habeva normal valores de glucosa sanguinee in stato jejun sed anormalitates in le tolerantia pro glucosa, esseva alimentate con un dieta de base supplementate—in le caso del animales experimental—

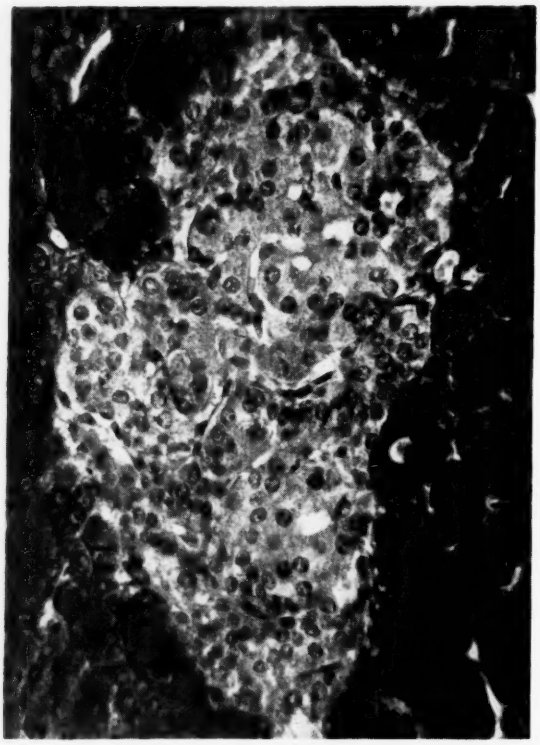

FIG. 8. Islet of Langerhans from a tolbutamide-treated alloxan "subdiabetic" rat showing characteristic beta cells rich in granulation. Alpha cells can be seen at the periphery and in the center of the islets. Gomori stain X 400.

de tolbutamida amontante a duo pro cento del total. Post inter dece-duo e dece-cinque menses de iste regime, le tolerantia pro glucosa esseva significativemente melior in le rattos tractate con tolbutamida que in le rattos recipiente le dieta de controlo. A iste tempore, le insulas del animales de controlo esseva marcatemente disgranulate, durante que illos del animales tractate con tolbutamida presentava un apparentia normal. Es formulate le conclusion que le perdurative administration de tolbutamida non age como causa sed possibilemente como factor preventive in le deterioration additional del function pancreatic de rattos in que le reserva pancreatic es reducite.

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The distinct disadvantage of excess weight in persons with various types of impairments shown by the Build and Blood Pressure Study, 1959, is confirmed by clinical investigations and earlier insurance studies. For example, the prognosis for insured persons with a history of asthma or bronchitis was considerably less favorable among those who were overweight than among those of average weight. Overweights with elevated blood sugar just below the normal limit and those with glycosuria are more likely to become diabetic than average or underweight persons with these impairments. The mortality of overweights with elevated blood sugar is higher than that for lighter weight people primarily because of increased death rate from cardiovascular disease and diabetes.

The present study underscores the desirability of weight control in persons with various types of impairments, even if they are of relatively minor significance. Although overweight usually is not the cause of the impairment, weight reduction will often decrease the adverse effects of the impairment. It is particularly important for overweights to have periodic health examinations so that defects may be detected and, if possible, corrected early.

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Human Serum Insulin-like Activity as Determined by a Rat Adipose Tissue Bio-assay Method

Paul M. Beigelman, M.D., Los Angeles

It has been demonstrated that a very sensitive insulin assay may be obtained by measuring glucose uptake of rat epididymal adipose tissue.[1-3] This method is characterized by an increase of glucose uptake directly proportional to insulin concentration in the range of 1 to 100 micro-units of insulin/ml.[4]

The present report is concerned with studies of sera from forty-seven nondiabetic humans. Particular emphasis is given to the effects of dilution and to changes in serum insulin-like activity (ILA) after eating. The present investigation indicates that this assay method is sufficiently sensitive to detect readily serum ILA in specimens diluted 1/4 to 1/10. However, this assay, at present, lacks precision.

METHODS

The technic of insulin bio-assay employing glucose uptake by rat epididymal adipose tissue has been described in some detail.[4] The method is somewhat improved by placing each rat in an individual cage with unrestricted access to food.

Blood specimens were secured either from subjects in the fasting state and at various intervals following ingestion of 100 gm. of glucose, or at random, particular attention being given to the time intervening since the subject last ate. All subjects were in presumably good health, and were mainly males in the twenty to forty age group. After venesection, the blood was permitted to clot, the serum was separated, and the assays were usually performed within the following one to two days.

Results have been calculated as the difference between the glucose uptake (mg. glucose/gm. adipose tissue), computed as log 10, of any individual serum and the corresponding mean glucose uptake of nonserum controls incubated with adipose tissue from the same rat. A mean value of multiple determinations for each serum specimen is presented, with significance ("P") calculated by the Student "t" test.

RESULTS

Random specimens of normal human blood were collected, the sera pooled, and varying dilutions of the pooled sera assayed. Insulin-like activity diminished in rough proportion to dilution (figure 1). Marked activity was demonstrated for undiluted serum ($P<.001$) and serum diluted 1/4 ($P<.01$). There was equivocal activity at 1/10 dilution ($P>.05,<.1$) and insulin-like activity of serum diluted 1/100 was significantly lower than that of corresponding nonserum controls. Previous studies in this laboratory have demonstrated that the addition of human serum albumin to the incubating medium is not associated with acceleration of glucose uptake by the epididymal fat pad.[5]

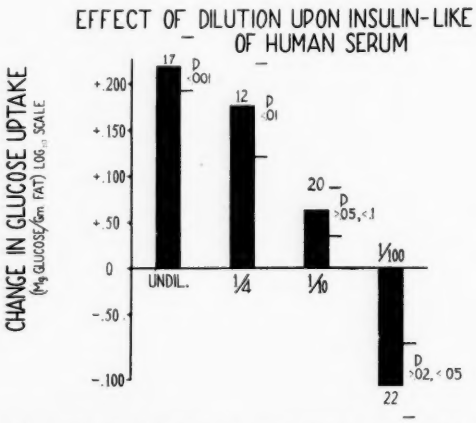

FIG. 1. Effect of dilution of serum upon glucose uptake by rat epididymal adipose tissue. Serum samples tested included no dilution, 1/4, 1/10 and 1/100 dilutions. Glucose uptake figures (log 10) are presented as differences between glucose uptakes obtained with serum in medium and without serum in medium, incubated with adipose tissue from same rat. Heights of bars represent mean change in glucose uptake. Brackets indicate S.E.M. Numbers above bars are numbers of determinations. P values, from Fisher's Tables, refer to significance of difference from buffer control.

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From the Department of Medicine, University of Southern California Medical School and the Los Angeles County Hospital, Los Angeles, California.

Serial determinations of serum insulin-like activity were made in the normal fasting state and at various intervals following oral ingestion of glucose or a meal. No consistent variations in serum ILA appeared which could be readily ascribed to the type of feeding the subject received. The sera were diluted 1/4 and 1/10. At both dilutions, it appears that there was little or no ILA present in the fasting state or up to 30 minutes postprandially, while significant serum ILA was evident thereafter (table 1, figures 2 and 3).

Considerable variation was evident even for serum samples from any one individual. In part, this could be attributed to the variability of the method, as indicated by the wide range of glucose uptake determinations

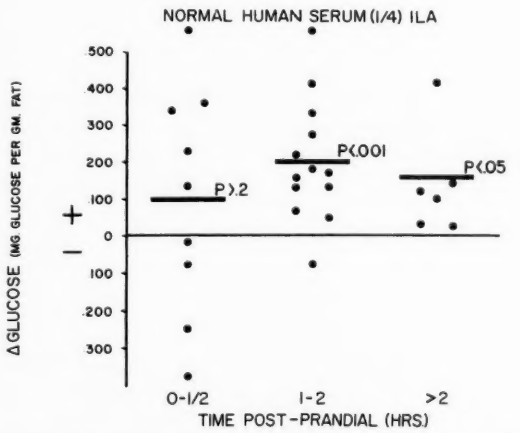

FIG. 2. Relationship of serum (diluted 1/4) insulin-like activity to time (hours) after eating meal or ingesting glucose. Each point represents insulin-like activity of a single serum. This is calculated as the mean difference of glucose uptake between medium with serum and medium without serum, incubated with adipose tissue from the same rat.

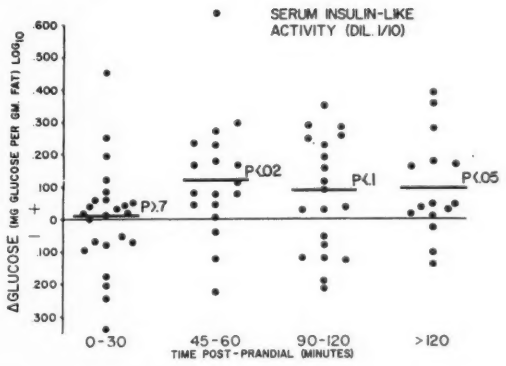

FIG. 3. Relationship of serum (diluted 1/10) insulin-like activity to time (minutes) after eating meal or ingesting glucose. Each point represents insulin-like activity of a single serum. This is calculated as the mean difference of glucose uptake between medium with serum and without serum, incubated with adipose tissue from the same rat.

obtained with each serum. However, the most important single factor influencing the serum insulin-like activity appears to be the length of time intervening between ingestion of glucose or food and venesection. There is also some association between serum glucose levels and insulin-like activity. Serum glucose levels greater than 140, occurring in eight instances, were never associated with measurable serum (diluted 1/10) ILA. These were generally obtained fifteen to thirty minutes postprandially. Conversely, serum glucose levels below 70 mg. per 100 ml. were associated with the presence of measurable activity in six of eight samples tested at a 1/10 dilution. These were specimens obtained one to three hours after meals.

One subject (BEI) was tested for serum insulin-like activity (dilution 1/10) seven times over about eight-

TABLE 1

Effect of time interval after eating upon serum insulin-like activity

Postprandial time interval	Serum dilution	Number of sera tested	Mean change log 10	Significance "P" of difference from		
				Buffer control	Fasting serum	15-30 min. Postprandial serum
Fasting	1/4	6	+.129	>.4	—	—
15-30 min.	1/4	3	+.047	>.6	>.6	—
60 min.	1/4	4	+.177	>.02,<.05	>.7	>.2,<.3
120 min.	1/4	7	+.248	>.01,<.02	>.4	>.1,<.2
150-180 min.	1/4	5	+.189	>.02,<.05	>.7	>.2,<.3
Fasting	1/10	10	+.003	>.9	—	—
5-30 min.	1/10	15	—.017	>.6	>.8	—
45-60 min.	1/10	19	+.113	>.01,<.02	>.2,<.3	>.01,<.02
90-120 min.	1/10	20	+.071	>.05,<.1	>.3	>.05,<.1
150 min. or greater	1/10	15	+.106	>.02,<.05	>.2,<.05	>.02,<.05

TABLE 2

Variations in serum (dil. 1/10) insulin-like activity of a single individual (BEI)

Date	Interval of time postprandial	Number of determinations	Blood glucose (mg. per 100 ml.)	Mean change*	Significance "p"‡
5/19/58	½ hr.	7	96	+.226	<.01
7/18/58	Fasting	8	104	+.251	<.01
	½ hr.	6	147	+.002	>.9
	1 hr.	15	76	+.180	>.05,<.1
	2 hr.	8	53	(—.122)†	(>.4)†
9/16/59	1½ hr.	6	102	+.250	<.01
	2 hr.	4	81	+.206	>.02,<.05
3/23/59	2 hr.	5	—	+.010	>.8
4/27/59	1½ hr.	8	—	(—.093)†	(>.1,<.2)†
10/31/59	Fasting	9	105	+.133	>.1,<.2
	15 min.	8	135	+.033	>.7
	30 min.	9	125	+.094	>.1,<.2
	1 hr.	9	83	+.164	>.02,<.05
	2 hr.	8	86	+.190	<.01
	3 hr.	19	68	+.179	<.001

*Mean change of glucose uptake as compared with equivalent nonserum control values—mg. glucose/gm. adipose tissue (log 10).
†Parentheses enclose figures indicating decrease of glucose uptake.
‡Refers to significance of difference from buffer control.

een months (table 2). Multiple sera for two of these assays were acquired in the course of oral glucose tolerance tests. Five of the seven tests demonstrated significant serum insulin-like activity. Each glucose tolerance test was characterized by significant insulin-like activity in one or more serum specimens.

Sera appear to have greater insulin-like activity at 1/4 than at 1/10 dilution. This is consistent with the results of testing varying dilutions of pooled sera.

DISCUSSION

In this study serum dilution appears to be associated with a corresponding, fairly proportional, diminution in glucose uptake. These results do not parallel those obtained with the rat diaphragm assay technic, which were characterized by comparatively little change in glucose uptake consequent to dilution, particularly in the range 1/2 to 1/4.[6,7] Also, it is of possible importance that the present report indicated diminution of glucose uptake in the presence of serum diluted 1/100 significantly less than that of corresponding media containing 0.2 per cent albumin. This may reflect the need for further effort to define more fully the activity of protein "control" material, including albumin and gelatin. Alternately there may be important shifting of the balance between complex insulin-like and anti-insulin effects of whole serum associated with degree of serum dilution.

Vallance-Owen[8] and Seltzer and Smith,[9] employing the rat diaphragm method, and Baird and Bornstein,[10] utilizing blood glucose changes in the anesthetized adrenalectomized mouse, noted marked increase of circulating human insulin-like activity following feeding. This study appears to confirm these observations.

The marked individual variations noted in this study while, in part, a reflection of the inherent variability of the method, also suggest true differences between human subjects. It has been emphasized that this bio-assay measures the total "insulin-like" effect, which is the total balance between circulating insulin, insulin-like factors, and anti-insulin substances.[8] A preponderance of anti-insulin factors at certain times may explain, in part, the occurrence of the occasional significant negative glucose uptake figures obtained in this study.

No attempt is being made in this investigation definitively to relate glucose uptake figures to quantitative insulin levels. Rough estimations, based upon the results of testing serum diluted 1/4, suggest an effective mean normal level of about 100 μU/ml. in the fasting state and 400 μU/ml. one to two hours postprandially.

SUMMARY

Normal human sera were assayed by a method utilizing glucose uptake by adipose tissue. Maximum insulin-like activity appeared to occur one to two hours postprandially. Considerable individual variation was a notable feature. Decrease of insulin-like activity was demonstrated as a consequence of diluting pooled whole serum.

SUMMARIO IN INTERLINGUA

Activitate Insulinoide de Sero Human, Determinate per un Methodo de Bio-Essayage con le Uso de Tissu Adipose de Ratto

Normal seros human esseva essayate per un methodo utilisante le acceptation de glucosa per tissu adipose. Le maximo del activitate insulinoide pareva occurrer inter un e duo horas post prandios. Considerabile grados de variabilitate individual esseva un notabile characteristica. Un reduction del activitate insulinoide esseva demonstrate como consequentia de un dilution de collationate sero total.

ACKNOWLEDGMENT

Portions of this study were supported by U. S. Public Health Service Grant No. A-1516 and a grant from the Diabetes Association of Southern California.

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Mortality of Overweights with Impairments

Some impairments generally considered to be of minor significance produce an unduly large increase in the mortality of overweights. This is one of the major findings of the Build and Blood Pressure Study, 1959, by the Society of Actuaries, which investigated separately the records of insured persons with minor impairments and of those without known minor impairments. The facts will be reviewed for males only, since their examinations for life insurance were more extensive and more thorough in most cases than those for females. For purposes of this study, a minor impairment is one which would not bar the applicant from obtaining standard insurance.

Overweight men with moderate elevation of blood pressure experienced a mortality nearly 60 per cent above that for overweights with no known minor impairments. For overweights with small or moderate amounts of albuminuria, the mortality was increased about 30 per cent; for those with other genitourinary disorders (such as kidney stone) and for those with a history of nervous disorders (chiefly psychoneurosis) the increase was about 15 per cent. Among overweight men with a family history of early cardiovascular-renal disease—considered to be a minor impairment for purposes of this study—the mortality was 35 per cent higher than for overweights of corresponding degree without known minor impairments.

The mortality among the overweight men with moderate elevation of blood pressure according to age at issue and degree of overweight is clearly shown. Not only is the mortality of these men consistently higher than that for all standard risks, but the excess is also quite large when compared with the mortality for men in the corresponding weight group without known minor impairments. The relatively small rise in the mortality ratios with increase in degree of overweight reflects, in part at least, the careful selection of the more obese applicants with moderately elevated blood pressure.

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The Effect of Denervation and Insulin on the Penetration of D-xylose into Rat Hemidiaphragms

Maria Gordon Buse, M.D., and John Buse, M.D.,
with the technical assistance of Eleanor Porter Sanders, B.S., Charleston, S.C.

One of the most characteristic actions of insulin is the stimulation of the utilization of glucose by muscle. Gemmill and Hamman showed in 1941 that the glucose uptake of the isolated rat diaphragm is enhanced by the presence of insulin in vitro. The factors that regulate the entrance of glucose into this tissue and the mechanism of the action of insulin on permeability to sugars have been extensively studied and recently reviewed.[1]

Previous work indicated[2] that rat hemidiaphragms decreased their responsiveness to insulin shortly after denervation. Their ability to respond to insulin by increasing the uptake of glucose in vitro was impaired, while no defect in the binding of insulin could be demonstrated using I^{131}-labeled insulin.

In the light of present concepts regarding the action of insulin the failure of the paralyzed diaphragm to respond to insulin with the expected increase in glucose uptake could be due to a "defect" in the transport system across the cell membrane, or to decreased intracellular metabolism of glucose resulting in a secondary block of the transport system, or to a combination of both processes.

The present experiments were designed to test the integrity of the transport system across the cell membrane of denervated rat hemidiaphragms. The penetration of *d*-xylose was studied because the transport of this sugar into muscle cells is accelerated by insulin. *D*-xylose is not appreciably metabolized by the isolated rat diaphragm and thus accumulates in the intracellular water under the influence of insulin.[1,3,4]

In this study the amount of *d*-xylose accumulating in the diaphragm after incubation was expressed as the "xylose space" which is that fraction of the volume of the tissue, which appears to contain fluid of the same xylose concentration as the medium. Interpretation of these results in terms of the distribution between the intracellular and extracellular compartment involves the estimation of the volume of the extracellular fluid. The volume of distribution of the thiosulfate ion was selected as representative of the extracellular space, and xylose was considered to be in the intracellular water when its volume of distribution exceeded that of thiosulfate. The thiosulfate and xylose spaces were determined in separate experiments in vitro, as the presence of thiosulfate has been reported to increase the penetration of *d*-xylose into the diaphragm under in vitro conditions.[4] When the thiosulfate space was measured, *d*-xylose, glucose and insulin were present in the same concentrations in the medium as in the parallel experiments without $Na_2S_2O_3$.

In experiments where the penetration of *d*-xylose into paralyzed and control hemidiaphragms was compared in the living animal, the thiosulfate and xylose "spaces" were determined simultaneously.

MATERIALS AND METHODS

Animals. Male Wistar rats were used. In the in vitro studies the weight of the animals was between 80 and 120 gm.; in the in vivo experiments between 140 and 200 gm. In individual experiments the body weights were kept as similar as possible, generally within 10 gm. The rats were fasted sixteen to twenty hours prior to the experiments.

Operative procedures. Unilateral phrenicectomies were performed one to eight days prior to the studies. Under light ether anesthesia the phrenic nerve was severed in the neck after identification by stimulation with faradic current. In each experiment equal numbers of right and left phrenicectomized animals were used.

In vitro experiments. The animals were killed by a blow on the head. The two hemidiaphragms (one paralyzed, the other control) were excised separately and placed in buffer solution (at 15° C.) for five to ten minutes. They were gently blotted and transferred to

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From the Departments of Medicine and Biochemistry, Medical College of South Carolina, Charleston, South Carolina.

tared Warburg vessels containing 2 ml. of medium. The weight of the tissues prior to incubation was determined by reweighing the vessels on a "Right-a-Weigh" analytical balance to the nearest 0.1 mg. The vessels were gassed for five minutes with 95 per cent O_2 and 5 per cent CO_2, stoppered and incubated for sixty minutes in a Warburg apparatus, with shaking (80 cycles/min.) at 37.4° C. At the end of the incubation period the vessels were chilled and the hemidiaphragms were rinsed in buffer for fifteen seconds. In some experiments the tissues were rapidly reweighed prior to extraction.

Incubation medium. Gey and Gey[5] balanced salt solution ($NaHCO_3$ buffer) plus *d*-xylose (3 mg./ml.) with or without added glucose (3 mg./ml.). Insulin* when present in the medium was at a concentration of 10^{-3} U./ml.; $Na_2S_2O_3$ when present: 10 μ Mol./ml. medium.

Chemical analysis. D-xylose: Each hemidiaphragm was extracted with 1 ml. of water for thirty minutes in a water bath at 100° C. This procedure gives complete extraction. The extract was deproteinized with copper sulfate and sodium tungstate[6] and *d*-xylose determined photometrically by the *p*-bromoaniline method of Roe and Rice.[7] Suitable aliquots of medium were deproteinized in the same way and analyzed for *d*-xylose.

Total water and thiosulfate. These determinations were carried out on the same sample of muscle. The tissues were extracted with 20 ml. of water at room temperature, for one hour, while bubbling the flasks to insure agitation. The tissues were then dried in an oven at 110° C. for two hours and reweighed. The water extracts were deproteinized with sodium tungstate and sulfuric acid[8] and thiosulfate in the filtrate assayed iodometrically.[8] Samples of medium were diluted, deproteinized and analyzed identically. In each experiment four hemidiaphragms were used as muscle blanks. These were incubated in identical media but without the addition of thiosulfate. Intact and denervated hemidiaphragms, with or without insulin, yielded identical and reproducible blanks.

In vivo experiments. Under light ether anesthesia *d*-xylose (0.8 gm./100 gm. body weight) was injected into the saphenous vein of unilaterally phrenicectomized, fasted rats. *D*-xylose was prepared as a 30 per cent solution, of which 3 to 4 ml. was administered within two minutes. The animals tolerated the procedure well and woke up from the anesthesia within a few minutes. Half of the rats were given intravenous insulin with the *d*-xylose injection, in the dose of 0.05 to one unit of insulin/rat. Forty-five minutes later the animals received 1 ml. of 1 N $Na_2S_2O_3$ I.V. One hour after the *d*-xylose injection, blood was drawn from the aorta into a heparinized syringe and the two hemidiaphragms excised separately. The blood was centrifuged and the plasma removed for chemical analysis. The hemidiaphragms were dipped in saline, gently blotted, cut in half and the two portions weighed separately. One half was analyzed for *d*-xylose, the other for total tissue water and $Na_2S_2O_3$ content, as described in the preceding paragraph. Plasma aliquots of 0.2 ml. were diluted, deproteinized, and their *d*-xylose and thiosulfate content determined. In each experiment one rat did not receive $Na_2S_2O_3$ and its plasma and hemidiaphragms were used as blanks of the corresponding thiosulfate determinations.

Presentation of data.

$$\text{Tissue water (ml./100 gm.)} = \frac{\text{wet wt.} - \text{dry wt.}}{\text{wet wt.}} \times 100$$

$$D\text{-xylose and thiosulfate spaces (ml./100 gm.)} = \frac{\text{muscle content (mg./gm. tissue wet wt.)}}{\text{medium concentration (mg./ml.)}} \times 100$$

The concentration of *d*-xylose in the intracellular water (mg./ml.):

$$Ci = \frac{Cd/Tw - Cm\ (St/Tw)}{1 - (St/Tw)}$$

where Cd is the concentration of *d*-xylose in the diaphragm (mg./100 gm. wet weight), Cm the concentration of *d*-xylose in the medium (mg./ml.), Tw the tissue water (ml./100 gm. wet weight), and St the thiosulfate space (ml./100 gm. wet weight).

In the tables mean values are presented ± the standard error of the mean. The number of observations are indicated in brackets.

RESULTS

(1) *In Vitro Experiments.*

(*a*) *Tissue water.* The mean total water content prior to incubation of sixty nondenervated control hemidiaphragms was 80.4 ± 0.2 per cent. This is slightly higher than that reported by other investigators[3,4] and may be due to the fact that younger animals were used. Denervation did not change significantly the total water content of the hemidiaphragms during the first two days after phrenicectomy (80.5 ± 0.3 per cent on the first and 80.7 ± 0.9 per cent on the second day). Three days after denervation there was a significant increase in the total water content of denervated hemidiaphragms, which rose to 83.0 ± 0.2 per cent of the wet weight. An average increase of 2 per cent in the wet weight of

* Five-times recrystallized zinc insulin, courtesy of Dr. F. B. Peck, Sr., Lilly Research Laboratories, Indianapolis, Indiana.

the hemidiaphragms was observed after one hour incubation in a medium containing glucose and *d*-xylose, at a concentration of 3 mg./ml. each. When only *d*-xylose (3 mg./ml.) was present in the medium, the mean increase in weight was 5 per cent. Neither denervation nor the presence of insulin could be shown to influence the degree of swelling during incubation in either medium.

(*b*) *Thiosulfate spaces.* Table 1 summarizes the results obtained when denervated and control hemidiaphragms were incubated in a medium containing *d-xylose* (3 mg./ml.). Table 2 summarizes experiments in which the medium contained 3 mg./ml. of glucose in addition to *d*-xylose. It will be noted that in both series of experiments the thiosulfate spaces were greater than those observed in vivo (table 3). Similar observations have been made by others[3,4] using the isolated "cut"-diaphragm preparation, in which a certain number of muscle fibers are necessarily damaged in the process of excising the muscle. When glucose was not present in the medium the thiosulfate spaces were greater (table 1) than in the presence of glucose and *d*-xylose (table 2). This confirms the observation of Kipnis and Cori[3] that the isolated diaphragm needs an energy source to maintain the cell membrane impermeable to thiosulfate. In both series of experiments the thiosulfate spaces of denervated hemidiaphragms were somewhat smaller than those of the controls. The difference was of statistical significance ($p < 0.05$) when glucose was also present in the medium (table 2). This might represent a shift of water from the extracellular to the intracellular space in paralyzed hemidiaphragms during incubation.

(*c*) *Xylose spaces.* Comparing the thiosulfate and xylose spaces in table 1 (medium: *d*-xylose 3 mg./ml.) it will be noted that the volume of distribution of *d*-xylose was larger than that of thiosulfate even in the absence of insulin. This indicates that intracellular penetration of *d*-xylose occurs in the absence of insulin, as has been reported.[1,3,4] Without insulin in the medium there was no difference in the penetration of *d*-xylose into denervated and control hemidiaphragms. Insulin increased the xylose spaces in controls and hemidiaphragms one and two days after denervation, by an average 30 per cent of the basal value. However, on the third day after denervation insulin had no effect on the xylose spaces of paralyzed hemidiaphragms while their intact pairs responded with the expected increase in the uptake of xylose ($p < 0.01$).

Table 2. In these experiments glucose and *d*-xylose (both at a concentration of 3 mg./ml.) were added to the medium. Again, the xylose spaces of all hemidiaphragms exceeded the volume of the extracellular water, indicating that *d*-xylose entered the cell even in the absence of insulin. Without insulin in the medium the

TABLE 1

The effect of denervation and insulin on *d*-xylose and thiosulfate spaces of hemidiaphragms in vitro

Parameter	Days after denervation	Insulin	Medium = *d*-xylose 3 mg./ml. Control	Paralyzed
D-xylose Space (Per cent of wet weight)	1	None	48.6 ± 4.0 (7)	42.0 ± 3.3 (7)
		10^{-3} U./ml.	64.7 ± 2.3 (7)	62.3 ± 2.0 (7)
		Insulin effect	+ 16.1 $p < 0.01$	+ 20.3 $p < 0.01$
	2	None	47.9 ± 2.7 (7)	49.3 ± 2.7 (7)
		10^{-3} U./ml.	60.4 ± 3.7 (7)	63.3 ± 2.0 (7)
		Insulin effect	+ 12.5 $p < 0.05$	+ 14.0 $p < 0.01$
	3	None	49.0 ± 2.0 (10)	50.7 ± 2.3 (10)
		10^{-3} U./ml.	*60.7 ± 2.3 (10)	*51.0 ± 1.1 (10)
		Insulin effect	+ 11.7 $p < 0.01$	+ 0.3 $p > 0.7$
Thiosulfate Space (Per cent of wet weight)	1 - 3	None	38.5 ± 2.1 (16)	35.2 ± 2.1 (16)
		10^{-3} U./ml.	39.4 ± 2.1 (16)	33.5 ± 2.9 (16)

* p value for difference between control and paralyzed < 0.01.

TABLE 2

The effect of denervation and insulin on *d*-xylose and thiosulfate spaces of hemidiaphragms in vitro

Medium = *d*-xylose 3 mg./ml. + *d*-glucose 3 mg./ml.

Parameter	Days after denervation	Insulin	Control	Paralyzed
D-xylose Space (Per cent of wet weight)	1	None	37.0 ± 2.0 (12)	33.3 ± 1.0 (12)
		10^{-3} U./ml.	*53.0 ± 1.3 (12)	*43.3 ± 2.0 (12)
		Insulin effect	+ 16.0 $p < 0.01$	+ 10.0 $p < 0.01$
	2	None	39.3 ± 3.0 (12)	36.0 ± 2.0 (12)
		10^{-3} U./ml.	*52.0 ± 2.7 (12)	*41.0 ± 1.3 (12)
		Insulin effect	+ 12.7 $p < 0.01$	+ 5.0 $p < 0.1$
	3	None	45.6 ± 3.0 (16)	37.7 ± 1.7 (16)
		10^{-3} U./ml.	*58.3 ± 3.0 (16)	*38.7 ± 1.1 (16)
		Insulin effect	+ 12.7 $p < 0.01$	+ 1.0 $p > 0.6$
Thiosulfate Space (Per cent of wet weight)	1 - 3	None	32.9 ± 1.1 (16)	29.9 ± 0.8 (16)
		10^{-3} U./ml.	30.1 ± 1.2 (16)	27.5 ± 0.9 (16)
		Insulin effect	— 2.8 $p < 0.05$	— 2.4 $0.05 < p < 0.1$

* p value for difference between control and paralyzed < 0.01.

TABLE 3

The effect of insulin and denervation on the total tissue water and extracellular space of hemidiaphragms

Parameter	Insulin U./rat	Controls	Paralyzed 1-8 days
Tissue water (Per cent of weight)	none	77.8 ± 0.28	79.1 ± 0.25
	0.05-1 U.	79.6 ± 0.32	80.0 ± 0.25
	insulin effect	+ 1.8 $p < 0.01$	+ 0.9 $p < 0.02$
Thiosulfate space (Per cent of weight)	none	20.9 ± 1.07	24.0 ± 0.94
	0.05-1 U.	17.6 ± 0.7	20.9 ± 0.79
	insulin effect	— 3.3 $p < 0.01$	— 3.1 $p < 0.01$

n = 36 in each group.

xylose spaces of denervated hemidiaphragms were not significantly different from those of the controls, although the volume of distribution of *d*-xylose tended to be somewhat smaller in paralyzed hemidiaphragms. The difference was of the same order of magnitude as that observed in the thiosulfate spaces.

Insulin increased the xylose spaces by an average 34 per cent of the basal value in control hemidiaphragms. This increase was of the same order of magnitude as that observed with *d*-xylose without glucose. However, when glucose was added to the medium the defect in the response to insulin of paralyzed hemidiaphragms was demonstrable earlier.

The xylose spaces of paralyzed muscles, when incubated with insulin were significantly smaller than those of the controls ($p < 0.01$) on the first, second, and third day after denervation. As in the preceding experiments paralyzed diaphragms failed to respond to insulin on the third day.

The bar graph (figure 1) represents volumes of distribution of *d*-xylose in the intracellular water of intact and denervated hemidiaphragms using the data from table 2. The percentage of intracellular water which equilibrated with the incubation medium was calculated as Ci/Cm × 100, where Ci is the concentration of *d*-xylose in the intracellular water, and Cm the concentration in the medium. In the absence of insulin *d*-xylose equilibrated with 7 to 27 per cent of the intracellular water of control hemidiaphragms. Insulin increased the volume of distribution of *d*-xylose to 44 to 57 per cent of the intracellular water in controls. Without insulin the intracellular xylose space of denervated hemidiaphragms was 7 to 14 per cent. Insulin was less effective in increasing the intracellular penetration of *d*-xylose in paralyzed hemidiaphragms than in controls. On the first day after denervation the intracellular xylose space of paralyzed diaphragms incubated with insulin was 29 per cent and was less than 20 per cent on the second and third day. These figures are presented

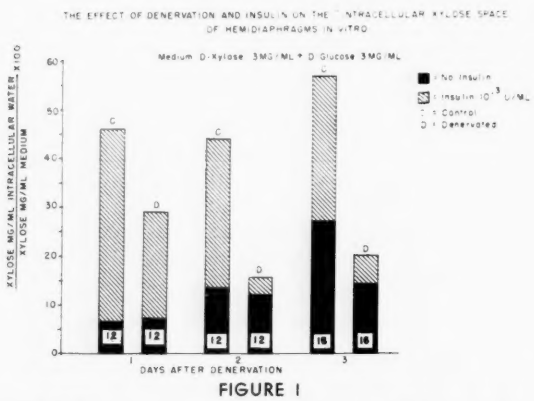
THE EFFECT OF DENERVATION AND INSULIN ON THE INTRACELLULAR XYLOSE SPACE OF HEMIDIAPHRAGMS IN VITRO
Medium D-Xylose 3MG/ML + D Glucose 3MG/ML
XYLOSE MG/ML INTRACELLULAR WATER / XYLOSE MG/ML MEDIUM x100
DAYS AFTER DENERVATION
C = Control
D = Denervated

FIGURE 1

graphically in order to emphasize that although there is some variation in the penetration of *d*-xylose in the absence of insulin, the striking feature of the denervated hemidiaphragm seems to be its decreased response to insulin. As *d*-xylose is not metabolized in the cell, this is interpreted as a defect in the response to insulin of the carrier system across the cell membrane.

(2) *Experiments in the living animal.*

The following studies compare the distribution of *d*-xylose in control and denervated hemidiaphragms in the living animal one hour after injection of the sugar. Insulin was injected simultaneously with *d*-xylose in half of the animals in a dose of 0.05 to 1 U. per rat. Preliminary analysis of the results showed that maximum penetration of *d*-xylose occurred within this dose range both into intact and denervated hemidiaphragms, and the data could be analyzed as a whole. Sodium thiosulfate was injected fifteen minutes prior to sacrificing the animals.

Table 3 records the total tissue water and thiosulfate spaces of denervated and control hemidiaphragms. As in the previous experiments denervation caused a small gradual increase in total tissue water. The maximum increase of 3 per cent was reached on the third day after denervation, after which time no further changes occurred. The increase in total water was accounted for by a parallel augmentation (3 per cent of the wet weight) of the thiosulfate spaces of denervated hemidiaphragms. These changes may be due to vasodilation of arterioles and capillaries secondary to denervation.[9]

After insulin injection a small but significant ($p < 0.01$) increase in total tissue water was observed in both denervated and control hemidiaphragms. This was accompanied by a parallel decrease ($p < 0.05$) in the thiosulfate spaces. It is concluded that after insulin administration water entered the muscle cells. This may be due either to the intracellular accumulation of *d*-xylose or to a direct action of insulin upon the transport of water.

Table 4 summarizes the results of the *d*-xylose determinations in intact and denervated hemidiaphragms. The values are expressed (a) as the total amount of *d*-xylose found in the tissues (mg./gm. wet weight) and (b) as the concentration of *d*-xylose in the intracellular water (mg./ml.). The first set of figures represents the direct measurements while the second expression is considered more meaningful in terms of tissue permeability. There is a close correlation between both groups of values as both insulin and denervation affected primarily the intracellular accumulation of *d*-xylose. In the following discussion of the data we will refer to the concentration of *d*-xylose in the intracellular water.

As the plasma concentrations fell during the one-hour duration of the experiments the results can not be interpreted in terms of the proportion of the intracellular water which equilibrated with the *d*-xylose concentrations in plasma.

The *d*-xylose concentrations found in the intracellular water of nonparalyzed hemidiaphragms increased more than threefold after insulin treatment rising from a mean value of 2.1 to 7.1 mg./ml. ($p < 0.01$ in all experiments). Insulin increased the concentration of *d*-xylose more in control than in denervated hemidiaphragms. At the time intervals studied (one to eight days after denervation) paralyzed hemidiaphragms of insulin treated rats contained approximately 70 per cent of the *d*-xylose concentration found in the controls, the difference being significant ($p < 0.01$) in all experiments.

Comparing the increments in *d*-xylose concentration after treatment with insulin it can be observed that on the average an additional 5 mg./ml. of *d*-xylose accumulated in the intracellular water of control hemidiaphragms under the influence of insulin and only 2.0 mg./ml. in their denervated pairs. The difference in "insulin effect" was significant in all experiments ($p < 0.01$).

On the first day after denervation the concentration of *d*-xylose was smaller in denervated than in control hemidiaphragms of rats that had not received insulin. On the second and third day after denervation more *d*-xylose accumulated in paralyzed muscles than in controls under the same conditions ($p < 0.01$). As this was not observed in the in vitro experiments, we attribute it to the experimental conditions prevailing in the living animal. A possible explanation is differences in blood flow, secondary to unilateral denervation. Similar observations

TABLE 4

The effect of denervation and insulin on the penetration of *d*-xylose* into rat hemidiaphragms in vivo

Days after denervation	Insulin 0.05 - 1 U./rat	Xylose content mg./gm. Tissue wet weight Control	Paralyzed	Xylose mg./ml. Intracellular water Control	Paralyzed
	—	2.8 ± 0.20 (14)	2.2 ± 0.19 (14)	2.6 ± 0.28 (14)	1.8 ± 0.25 (14)
1	yes	5.5 ± 0.40 (13)	†3.8 ± 0.22 (13)	6.8 ± 0.40 (13)	†4.2 ± 0.28 (13)
	d =	2.7 ± 0.45 p < 0.01	1.6 ± 0.29 p < 0.01	4.2 ± 0.48 p < 0.01	†2.4 ± 0.37 p < 0.01
	—	2.6 ± 0.18 (8)	†3.3 ± 0.14 (8)	1.8 ± 0.22 (8)	†3.0 ± 0.26 (8)
2	yes	5.6 ± 0.29 (7)	†4.4 ± 0.22 (7)	7.4 ± 0.44 (7)	†5.4 ± 0.39 (7)
	d =	3.0 ± 0.34 p < 0.01	†1.1 ± 0.26 p < 0.01	5.6 ± 0.49 p < 0.01	†2.4 ± 0.47 p < 0.01
	—	2.5 ± 0.22 (9)	†3.9 ± 0.23 (9)	1.9 ± 0.28 (9)	†4.2 ± 0.33 (9)
3	yes	5.4 ± 0.51 (10)	4.2 ± 0.35 (10)	7.0 ± 0.58 (10)	†4.7 ± 0.54 (10)
	d =	2.9 ± 0.55 p < 0.01	†0.3 ± 0.75 p > 0.6	5.1 ± 0.64 p < 0.01	†0.5 ± 0.63 p > 0.5
	—	1.9 ± 0.21 (4)	2.9 ± 0.22 (4)	1.6 ± 0.33 (4)	2.5 ± 0.54 (4)
8	yes	5.6 ± 0.42 (4)	4.3 ± 0.41 (4)	7.6 ± 0.36 (4)	†5.1 ± 0.57 (4)
	d =	3.7 ± 0.47 p < 0.01	1.4 ± 0.47 p < 0.05	6.0 ± 0.47 p < 0.01	†2.6 ± 0.78 p < 0.05
	—	2.7 ± 0.13 (35)	3.0 ± 0.15 (35)	2.1 ± 0.15 (35)	2.8 ± 0.22 (35)
Mean 1-8	yes	5.5 ± 0.19 (34)	†4.1 ± 0.15 (34)	7.1 ± 0.25 (34)	†4.8 ± 0.23 (34)
	d =	2.8 ± 0.22 p < 0.01	†1.1 ± 0.21 p < 0.01	5.0 ± 0.29 p < 0.01	†2.0 ± 0.32 p < 0.01

* *D*-xylose 0.8 gm./100 gm. injected i.v. with or without insulin one hour prior to autopsy.
† p value for difference between control and paralyzed < 0.01.
d = insulin effect.

were made in studies of the insulin binding capacity of denervated hemidiaphragms.[2] Paralyzed and control muscles bind the same amount of insulin-I^{131} in vitro but the former bind twice as much insulin as the controls when the hormone is injected I.V. three days after unilateral phrenicectomy.

DISCUSSION

The present studies indicate that paralyzed hemidiaphragms have an impaired response to insulin with respect to the transport of *d*-xylose into the cell. It has been recently demonstrated[1,10,11] that *d*-xylose and glucose compete in muscle for the same insulin responsive transport system. Therefore, it is probable that the diminished glucose uptake of paralyzed hemidiaphragms in the presence of insulin,[2] is determined at least in part by a decrease in the rate of transport of glucose.

The decreased effect of insulin on *d*-xylose transport could be observed on the first day after denervation only when glucose was added to the medium. Substitution of sodium pyruvate for glucose as an oxidizable substrate did not reproduce this effect (unpublished results). Three days after denervation the decreased effect of insulin on *d*-xylose transport could be demonstrated both in the presence and absence of glucose. Studies of glucose uptakes in denervated hemidiaphragms showed that the impairment of the response to insulin is gradually progressive; it could be observed twelve hours after denervation and was maximal on the third day.[2] As glucose is metabolized after penetration into the cell, relatively small differences in the rate of transport are reflected in measurable changes of glucose uptake, when transport is rate limiting. On the other hand, as *d*-xylose accumulates in the cell, a relatively small decrease in the rate of transport could be missed, if measurements are made close to the final equilibrium between the intracellular water and the medium.[1,3] This may explain why the decreased response to insulin was observed only on the third day after denervation, when *d*-xylose alone was the substrate. The decreased effect of insulin on the accumulation of *d*-xylose was demonstrable earlier in the presence of glucose, probably due to competition between glucose and *d*-xylose for a "reduced" carrier system.[1,10,11]

In the experiments presented the impaired penetration of *d*-xylose into denervated hemidiaphragms could be demonstrated only when insulin was present in the medium or injected into the animal. Similarly, paralyzed hemidiaphragms consumed less glucose than controls only when insulin was added to the medium or

injected in vivo.[2] This could indicate that nervous stimuli or muscular work are necessary for the optimal action of insulin upon the transport system in muscle and do not influence the carrier system itself.

In the in vitro experiments the "control" hemidiaphragms were working up to the time of incubation, and in the in vivo studies rhythmically contracting "control" hemidiaphragms were compared to their denervated pairs. It has been shown recently that anoxia stimulates the transport of sugars in muscle[4,10-12] and some degree of anoxia occurs in muscle during exercise. However, the acceleration of transport observed during anaerobic incubation is reversed very rapidly to the aerobic rate in the presence of oxygen.[10] In the present studies, control and denervated hemidiaphragms were incubated for one hour in a 95 per cent O_2 atmosphere, which makes it highly improbable that the observed differences in transport were due to the oxygen debt incurred by the control diaphragms.

Randle and Smith suggested recently[4,12] that the entry of glucose and related sugars into muscle cells is restrained under basal conditions by a process dependent upon the supply of energy rich phosphate. They also indicated that insulin may stimulate the transport of sugars by restricting the access of energy-rich phosphate to the process concerned with the regulation of glucose entry. Continuing this line of speculation nervous stimuli or muscular work would influence this second step in the regulation of glucose metabolism either by supplying a factor necessary for the interaction of insulin with the high energy phosphates or by removing an inhibitor of this process. The denervated muscle may provide a valuable tool for the assessment of the factors that regulate glucose utilization by muscle and eventually the mode of action of insulin.

SUMMARY

The penetration of *d*-xylose into rat hemidiaphragms was studied one to eight days after unilateral denervation.

(1) Intact and paralyzed hemidiaphragms were incubated in buffer containing 3 mg./ml. *d*-xylose with or without added insulin (10^{-3} U./ml.). *D*-xylose was considered intracellular when its volume of distribution (*d*-xylose space) exceeded that of the extracellular water (thiosulfate space).

No significant difference was observed between the *d*-xylose spaces of control and denervated hemidiaphragms in the absence of insulin. In the presence of insulin denervated hemidiaphragms showed less accumulation of *d*-xylose than the controls ($p < 0.01$). The impaired response to insulin of paralyzed hemidiaphragms was demonstrable from the first day after denervation when glucose (3 mg./ml.) was also present in the medium. On the third day after denervation insulin failed to increase the intracellular penetration of *d*-xylose both in the presence and absence of glucose.

(2) *D*-xylose (0.8 gm./100 g.b.w.) was injected with or without insulin (0.05-1 U./rat) to unilaterally phrenectomized fasted rats. The *d*-xylose content of plasma and hemidiaphragms was measured one hour after the injection and the $Na_2S_2O_3$ spaces determined simultaneously. One to eight days after denervation the intracellular concentration of *d*-xylose was significantly lower in paralyzed hemidiaphragms of rats treated with insulin than in their nonparalyzed pairs ($p < 0.01$).

It is concluded that muscular exercise or nervous stimuli are necessary for the optimal response to insulin of the transport system across the cell membrane in muscle.

SUMMARIO IN INTERLINGUA

Le Effecto de Denervation e de Insulina Super le Penetration de D-Xylosa a in le Hemidiaphragma del Ratto

Le penetration de *d*-xylosa in le hemidiaphragma del ratto esseva studiate inter un e octo dies post denervation unilateral.

1. Intacte e paralysate hemidiaphragmas esseva incubate in tampon continente 3 mg/ml *d*-xylosa con o sin le addition de insulina (10^{-3} u/ml). Le *d*-xylosa esseva considerate como intracellular quando su volumine de distribution (le spatio de *d*-xylosa) excedeva illo del aqua extracellular (spatio de thiosulfato).

Nulle significative differentias esseva observate inter le spatios de *d*-xylosa in le hemidiaphragmas de controlo e illos in le hemidiaphragmas denervate in le absentia de insulina. Tamen, in le presentia de insulina, le denervate hemidiaphragmas monstrava un minus marcate accumulation de *d*-xylosa que le hemidiaphragmas de controlo ($p < 0{,}01$). Le reducite responsa a insulina del parte del paralysate hemidiaphragmas esseva demonstrabile a partir del prime die post le denervation quando glucosa (3 mg/ml) esseva etiam presente in le medio. Le tertie die post le denervation, insulina non promoveva le penetration intracellular de *d*-xylosa, sin reguardo a si o non glucosa esseva presente.

2. *D*-xylosa (0,8 g per 100 g de peso corporee) esseva injicite sin o con insulina (0,05 a 1 u/ratto) a unilateralmente phrenicectomisate rattos in stato jejun. Le contento de *d*-xylosa del plasma e del hemidiaphragmas esseva mesurate un hora post le injection, e le spati-

os de $Na_2S_2O_3$ esseva determinate simultaneemente. Un a octo dies post le denervation, le concentration intracellular de *d*-xylosa esseva significativemente plus basse in paralysate hemidiaphragmas de rattos tractate con insulina que in lor non-paralysate pares ($p < 0{,}01$).

Es concludite que exercitio muscular o stimulos nervose es necessari pro le responsa optimal a insulina del parte del systema de transporto a transverso le membrana cellular in musculo.

ACKNOWLEDGMENT

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Overweights Benefit from Weight Reduction

New evidence that weight reduction has a favorable effect on the longevity of overweights is provided by the Build and Blood Pressure Study, 1959, recently completed by the Society of Actuaries. One aspect of the study dealt with the mortality experience of policyholders who were charged higher than standard premiums solely because they were overweight at the time they were insured but who later qualified for insurance at standard rates because they had reduced their weight sufficiently. The mortality of these insured was significantly lower than that for the entire group of policyholders in their original overweight class. This study confirms the results of a similar investigation made some years ago by the Metropolitan Life Insurance Company.

Adequate data for detailed analysis were available only for male risks, but the small experience for overweight women who reduced indicated that their mortality likewise was more favorable than that for the entire group of women originally charged higher than standard premium rates on account of overweight.

Overweight men who reduced experienced practically the same mortality as standard risks for a period of years following weight reduction. This was true for each broad age group and for all height classes. The mortality record of the men who reduced was most favorable in the years immediately following removal of the substandard rating, but less so thereafter; this upward trend in mortality was characteristic of all overweights. The mortality of the men who reduced nevertheless remained consistently below that of overweight men who continued to pay extra premiums.

The experience for men was analyzed further according to weight class: those averaging about 25 per cent above average weight and those about 35-40 per cent overweight at the time the insurance was issued. In both groups the mortality after weight reduction was close to the average for all standard risks. In each of the weight classes, the mortality of those who reduced was well below that for the group from which they were drawn. The decrease in mortality was especially large for the men markedly overweight.

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Studies on the Physical, Immunological and Biological Properties of Insulin Conjugated with Fluorescein Isothiocyanate

Demetreos N. Halikis, M.D., and Edward R. Arquilla, M.D., Ph.D., Los Angeles

A physiologically active preparation of fluorescent insulin would help in localizing the site of action of insulin. In an attempt to prepare a physiologically active form of labeled insulin, a fluorescein isothiocyanate derivative of insulin was prepared, and its biological, immunological, and physical properties were investigated.

The localization of dye-labeled protein in tissue has been previously demonstrated by Sabin[1] with R-salt-azobenzene-azo-egg albumin. Coons and others[2-4] were able to localize antigens in tissue at a cellular level with fluorescein isocyanate-labeled antibodies. Recently Riggs[5] modified the method of conjugating fluorescein to protein by employing the isothiocyanate of fluorescein. This compound is more stable than fluorescein isocyanate and the protein is not denatured to any detectable degree during the process of conjugation.[6]

The use of radioautographs to determine the localization of I^{131}-labeled insulin[7,8] has been investigated. The radioautographic method of localizing radioactive material is indirect, consequently the pictures are blurred and as a result lack definition. It is hoped that better definition of the anatomical site of action of insulin at a cellular level can be achieved with fluorescent insulin.

The following experiments demonstrate that fluorescent insulin has decreased biological activity and decreased ability to react with antibodies to crystalline insulin. Fluorescent insulin has two components by paper electrophoresis, each of which migrates faster than crystalline insulin. Its fluorescence is augmented in the presence of added serum protein.

METHODS AND MATERIALS

Preparation of fluorescein isothiocyanate-conjugated insulin: Fifty mg. of beef zinc crystalline insulin* (Lot no. 535664) was added to each of two 25 ml. volumetric flasks. The insulin was dissolved with 5 ml. of 0.01 N NaOH and then buffered to *p*H 7.4 with 5 ml. of 0.22 M PO_4 buffer. To one flask, approximately 23 mg. of fluorescein isothiocyanate (Sylvana Chemical Co., Orange, New Jersey) was added. The final volumes of both flasks were adjusted to 25 ml. with an acetone-saline-bicarbonate buffered solution. The buffer was prepared by mixing 30 ml. of saline, 6 ml. of acetone, and 9 ml. of a 0.5 M HCO_3 buffer at *p*H 9.0, as described by Riggs.[5]

The flasks were agitated overnight in a 1° C. water bath. Aliquots (10 ml.) of the resultant mixtures were each dialyzed (Visking tubing, size 20/32) for five days against eight one-liter changes of saline containing 0.01 M phosphate buffer, *p*H 7.0.

Electrophoresis: A Spinco electrophoretic cell (Model R), veronal buffer (ionic strength 0.08 at *p*H 8.6), 3 mm. Whatman filter paper strips, and a Heathkit variable voltage regulated power unit were used. In all experiments the cell was loaded with eight paper strips and allowed to equilibrate at least two hours before applying the various preparations in volumes between 10 to 50 lambda with the Spinco sample striper. A 9.0 milliamp current, with a voltage between 80 and 110, was then immediately applied for a duration of sixteen hours. The strips were dried in a 110° C. hot air oven for thirty minutes and then stained during a five-hour period with a 0.1 per cent bromphenol blue[9] solution. The strips were then washed twice in a 5 per cent acetic acid solution for ten minutes and air dried fifteen minutes at 110° C. The intensity of the blue stain was augmented by exposing the dried stained paper strips to ammonium fumes.

Paper electrophoresis strips with fluorescent material were divided in half. One half was studied under ultraviolet light and the other half was stained with bromphenol blue. Preliminary experiments showed that staining with bromphenol blue quenched fluorescence.

Protein determination: Proteins were determined by the method of Lowry, Rosebrough, Farr and Randall.[10] The standard curve for the protein determination was established, using bovine crystalline insulin. The protein content of solutions containing fluorescent insulin and of

From the Department of Pathology, University of Southern California School of Medicine, Los Angeles, California.

*We wish to thank Dr. Otto K. Behrens, Eli Lilly and Company, for the generous supply of crystalline beef insulin.

control solutions containing crystalline insulin differ by a maximum of 5.7 per cent and 3.4 per cent from the theoretical concentrations (table 1).

TABLE 1

Recovery of fluorescein labeled and zinc crystalline beef insulin after extensive dialysis

	Fluorescein labeled insulin		Crystalline insulin	
Experiment No.	1	2	1	2
Original weight insulin (mg.)	21.1	20.0	22.0	20.0
Original volume (ml.)	11.0	10.0	11.0	10.0
Volume after dialysis (ml.)*	12.24	11.84	12.54	11.65
Theoretical conc. (mg./ml.)†	1.73	1.68	1.75	1.73
Observed conc. (mg./ml.)	1.80	1.65	1.65	1.69
Per cent recovered	104.0	97.5	94.5	98.5

*The volume after dialysis was determined by weight.
†The original quantity of insulin was divided by the final volume after dialysis.

Factors influencing fluorescence of fluorescein-labeled insulin: A Turner fluorometer (Model 110) was used to measure fluorescence. At any one of the four slit widths a linear relationship existed between the fluorescence emission readings and the concentration of fluorescein isothiocyanate or fluorescent insulin, with a range of about one log unit. It was possible to measure as little as 1×10^{-7} gm./ml. of fluorescent insulin or 1×10^{-9} gm./ml. of fluorescein isothiocyanate, with a maximum error of 5 per cent.

The fluorescence of fluorescein isothiocyanate and fluorescent insulin is affected by the *p*H of the diluent. Since the fluorescence of fluorescein approaches maximum at *p*H 8.5, all test solutions were diluted with a 0.025 M borate buffer, *p*H 9.0.

Extraneous fluorescence of sera and other compounds in the diluent were minimized by using a primary Eastman Kodak No. 47B filter and a secondary Eastman Kodak No. 2A-12 filter.

Immunological properties: Hemagglutination and cross hemagglutination inhibition experiments were performed, using the methods and materials described by Arquilla and Stavitsky.[11] Known amounts of crystalline and fluorescent insulin were preincubated with aliquots of each serial dilution of rabbit antiserum to crystalline insulin. In the control, saline was preincubated with the same dilutions of antiserum. Erythrocytes conjugated with crystalline or fluorescent insulin were then added to all the samples.

Biological properties: Fasted male Wistar strain rats, between 90 and 150 gm., were anesthetized with an intraperitoneal injection of 0.1 ml. of 6 per cent Nembutal per 100 gm. weight of animal. The tip of the tail was clipped and an initial blood sample (0.05 ml.) was taken. The tip of the tail was then cauterized and the test solution in a volume of less than 0.5 ml. was injected intravenously via the tail vein. One hour after injection of the test material a second sample was taken for estimation of blood glucose. Preliminary experiments with various doses of crystalline insulin and fluorescent insulin showed that the maximum depression in blood glucose occurred one hour following injection. Blood glucose was determined by the glucostat method.[12]

RESULTS

Electrophoretic properties: Simultaneous paper electrophoretic analyses were performed with dialyzed fluorescent insulin, nondialyzed fluorescent insulin, fluorescein isothiocyanate, crystalline insulin, and rabbit serum (figure 1).

Fluorescein isothiocyanate had at least three mobile components, whereas nondialyzed fluorescent insulin and dialyzed fluorescent insulin each had two (figure 1). Most of the fluorescent material migrated faster than crystalline insulin and rabbit serum albumin. The two components of dialyzed fluorescent insulin had less fluorescence than the two components of nondialyzed fluorescent insulin and the three components of fluorescein isothiocyanate. The faster of the two components of dialyzed fluorescent insulin was more faint, and less distinct, than the slower component. The migration of this fast component is equal to that of the fast component of nondialyzed fluorescent insulin and of the middle component of fluorescein isothiocyanate.

It therefore appears that by paper electrophoresis there are at least two components of fluorescent insulin. The characteristics of the individual components have not been studied.

Effect of protein on the fluorescence of fluorescein isothiocyanate and fluorescent insulin: Serial doubling dilutions of normal rabbit serum and immune rabbit serum were prepared. A 3 ml. aliquot of each dilution was mixed with 3 ml. of a 2×10^{-6} gm./ml. of fluorescent insulin in a 13 x 100 mm. Pyrex tube. The Pyrex test tubes were previously matched with a standard fluorescein isothiocyanate solution to within 0.5 per cent error. A second 3 ml. sample of each of the master dilutions of normal rabbit serum was mixed with a 1×10^{-7} gm./ml. of fluorescein isothiocyanate. Dilutions of normal rabbit serum to less than 1:6,400 augmented the fluorescence of fluorescent insulin (figure 2). Dilutions of normal rabbit serum to less than 1:6,400 had a small but distinct quenching effect on the fluorescence of fluorescein isothiocyanate. The fluorescence of insulin labeled with fluorescein was augmented when mixed with solutions of purified bovine serum albumin and purified human gamma globulin in concentrations exceeding 50

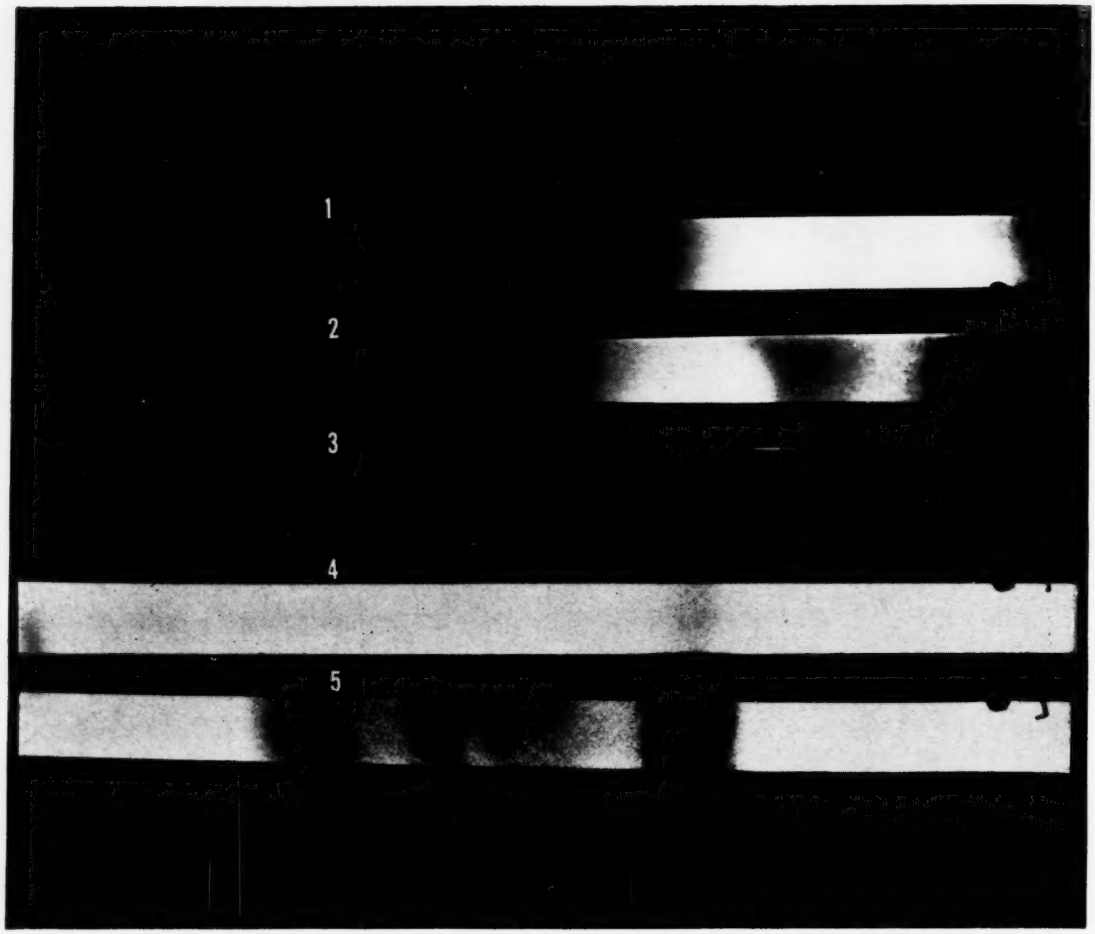

FIG. 1. Electrophoretic patterns of fluorescein isothiocyanate and fluorescent insulin. Strip 1. Fluorescein isothiocyanate (under ultraviolet light). Strip 2. Nondialyzed fluorescent insulin (under ultraviolet light). Strip 3. Dialyzed fluorescent insulin (under ultraviolet light). Strip 4. Crystalline bovine insulin. Strip 5. Normal rabbit serum.

μg./ml. It therefore appears that the fluoresence of insulin labeled with fluorescein is augmented in the presence of serum proteins, whereas serum proteins appear to quench the fluorescence of fluorescein isothiocyanate.

Immunological results: The immunological properties of dialyzed fluorescein isothiocyanate-conjugated insulin were compared with those of crystalline insulin by hemagglutination and cross inhibition of hemagglutination[11] (table 2).

Fluorescent insulin-sensitized sheep erythrocytes were agglutinated by lower dilutions (higher antibody concentrations) than sheep erythrocytes sensitized with crystalline insulin (table 2). This decreased titer with fluorescent insulin was probably not due to the differences in the degree of sensitization of erythrocytes, since optimal sensitization of the erythrocytes by fluorescent insulin and crystalline insulin was previously determined. Therefore, the decreased titer observed with fluorescent insulin-sensitized cells was probably due to the decreased ability of rabbit antibody against crystalline insulin to react with the fluorescent insulin that was conjugated to the erythrocytes.

No immunological difference was observed between fluorescent insulin and crystalline insulin in the cross inhibition experiments. Various quantities of dialyzed fluorescent insulin, dialyzed crystalline insulin, and crystalline insulin were preincubated with rabbit insulin antiserum prior to the addition of sensitized cells. No differences were noted in the degree of inhibition by the various insulin preparations (table 2). Preincubation of

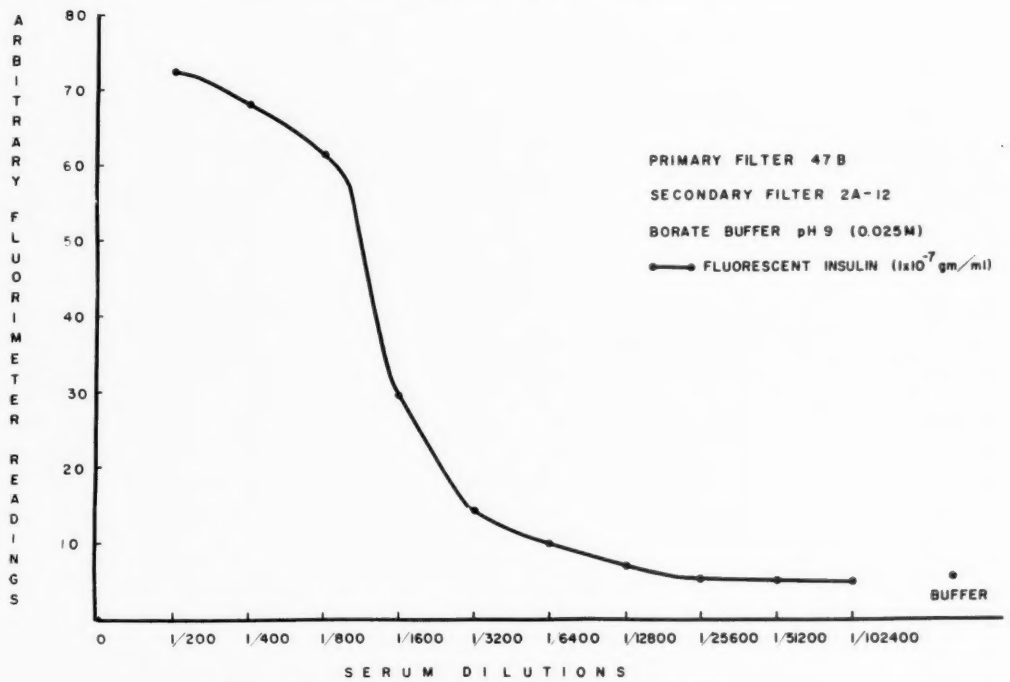

FIG. 2. Effect of serum on fluorescence of fluorescent insulin.

TABLE 2

Immunological properties of zinc crystalline beef insulin and dialyzed fluorescein labeled and crystalline beef insulin

Inhibition		Crystalline beef insulin sensitized sheep RBC	Fluorescein labeled insulin sensitized sheep RBC
Crystalline Insulin	50 γ	1/40*	<1/20*
	0.5γ	1/320	1/20
Dialyzed Insulin	50 γ	1/20	<1/20
	0.5γ	1/320	<1/20
Dialyzed fluorescein Labeled insulin	50 γ	1/40	<1/20
	0.5γ	1/320	<1/20
None		1/1,280	1/320

*Dilution of rabbit crystalline-insulin antiserum in 0.5 ml. which agglutinated 0.05 ml. of 2 per cent suspension of sensitized erythrocytes.

the various insulin preparations with rabbit anti-insulin serum inhibited the hemagglutination of fluorescent insulin-sensitized cells.

Therefore it appears that the agglutination of fluorescent insulin-sensitized cells by the antibody to crystalline insulin is decreased. This altered immunological activity of fluorescent insulin could not be demonstrated in the cross inhibition reaction.

Biological activity: The biological activity of dialyzed fluorescent insulin, dialyzed crystalline insulin, and crystalline insulin were evaluated.

Fluorescent insulin at two-dose levels did not reduce the blood glucose of rats to the same degree as similar dose levels of crystalline insulin (table 3). Statistical comparison of the per cent blood glucose response of rats injected with fluorescent insulin and crystalline insulin show a p value of less than 0.001. No difference was noted in the blood glucose response of rats injected with like doses of crystalline insulin and dialyzed insulin. It therefore appears that the reduced biological activity of insulin when conjugated with fluorescein isothiocyanate is not due to the conditions of the method of conjugation per se, but due to the effect of the fluorescein isothiocyanate on the insulin molecule.

The minimum response dose of fluorescent insulin was 2.2 μg. per 100 gm. weight of rat. Statistical comparisons of the blood glucose responses in this group and the controls given buffered saline showed a p value of less than 0.01. Preliminary studies, using small numbers of rats, showed that larger doses of fluorescent insulin caused a greater fall in blood glucose.

The mean per cent blood glucose drop in nine rats injected with 1.1 mg./100 gm. of fluorescent insulin was 12 ± 13.6 mg. per cent. The mean per cent blood glucose changes in one hour after the I.V. injection

TABLE 3

Hypoglycemic activity of fluorescein labeled insulin compared with either crystalline insulin or buffered saline*

		Mean per cent decrease in blood glucose		
I	Buffered saline	1		
II	Crystalline insulin 1.1 μg (0.03 units)	49		
III	Crystalline insulin 2.2 μg. (0.06 units)	66		
IV	Fluorescent insulin 1.1 μg.	12		
V	Fluorescent insulin 2.2 μg.	16		
	Significance of IV against I		P	0.4
	" IV " II		P	0.001
	" IV " III		P	0.001
	" V " I		P	0.01
	" V " II		P	0.001
	" V " III		P	0.001

*Adult white male Wistar strain rats between 90 and 150 gm. were anesthetized with Nembutal intraperitoneally. A preinjection blood sample was taken from the tail. The rats were then given either buffered saline, insulin or fluorescein conjugated insulin I.V. by tail veins in a volume less than 0.5 ml. One hour following injection another blood sample was taken.

of buffered saline in thirteen control rats was 1 ± 15.7 mg. per cent. The statistical evaluation of the differences of both groups of rats gave a p value of less than 0.4.

Fluorescent insulin had decreased biological activity as compared with crystalline insulin. There was no detectable effect on the biological activity of crystalline insulin when exposed to the conditions necessary to conjugate fluorescein isothiocyanate to insulin. It therefore appears that fluorescein isothiocyanate when conjugated to insulin reduced the biological activity of insulin.

DISCUSSION

It is hoped that fluorescent insulin with physiological activity equal to crystalline insulin may be prepared. Such a preparation would then be helpful in defining the anatomical locus of action of insulin at the cellular level. Stein and Gross[8] using I^{131}-labeled insulin were able to demonstrate the intracellular localization of insulin in heart, liver, and lung of rats. They could not conclusively localize insulin in striated muscle. Elgee, Williams, and Lee[7] previous to this, using I^{131} insulin, demonstrated the relative degree of localization of insulin in various organs and were not concerned with the localization of insulin at a cellular level.

It has been possible to localize antigens intracellularly with the fluorescent antibody technic.[3,4] Antigens have been labeled with dye and their localization has been determined at a cellular level. It is not known whether a hormone labeled with fluorescein would give a definitive localization of its site of action. A fluorescent insulin preparation would be useful to evaluate the site of action of insulin. It is essential that the characteristics of such a preparation be evaluated.

The physical, immunological, and biological properties of fluorescent insulin have been studied. Insulin conjugated with fluorescein isothiocyanate had at least two components by paper electrophoresis and its fluorescence was augmented by serum proteins. The biological activity of insulin was decreased and its immunological activity was altered.

Electrophoretic studies showed that the fast, faint, less distinct component of dialyzed fluorescent insulin was probably absorbed onto the paper during its migration. This is substantiated by the trailing of fluorescent material on paper electrophoretic strips. The slow component is more distinct probably because binding sites on paper had been saturated by the fast component.

The electrophoretic pattern of dialyzed fluorescent insulin had at least two components, each faster than crystalline insulin and insulin treated under the same conditions of conjugation. The electrostatic characteristic of fluorescent insulin is relatively more negative than crystalline insulin. This difference is probably due to the addition of negative fluorescein isothiocyanate molecules to insulin.

Electrophoretic studies of fluorescein isothiocyanate showed at least three mobile components. The fast component was probably conjugated to insulin. The amount of fluorescein isothiocyanate from the other two components that was conjugated to insulin has not been evaluated. Separation of the components of fluorescein isothiocyanate and their ability to conjugate with protein is being investigated.

The quantity of fluorescein removed by dialysis is difficult to determine since fluorescence decays with time. Therefore it was not possible to estimate the average number of molecules of fluorescein conjugated to a molecule of insulin. The fluorescent spectra of fluorescein was not shifted when conjugated with insulin nor when proteins were added to fluorescent insulin.

The intensity of fluorescence is augmented when serum proteins are added to solutions of fluorescent insulin. It appears that the fluorescence of fluorescein is quenched when conjugated to insulin. It is unlikely that the augmented fluorescence by protein was due to increased emission from free fluorescein molecules which were not quenched, since the fluorescence of free fluorescein isothiocyanate was quenched in the presence of added protein. It is probable that serum proteins, to some degree, reversed the quenching of fluorescein conjugated to insulin. It is likely that tissue proteins will also augment the fluorescence of fluorescent insulin.

Fluorescein apparently interferes with the antibody-antigen reaction when fluorescent insulin is attached to

sheep erythrocytes. It appears that in solution the spatial arrangement of the fluorescein molecule on insulin does not affect the immunological response of insulin to antibody to any measurable degree by hemagglutination inhibition. Specific rabbit crystalline-insulin antisera agglutinated sheep erythrocytes sensitized with fluorescent insulin to a lesser degree than erythrocytes sensitized with crystalline insulin.

The hemagglutination method cannot be used to evaluate the uniformity of labeling of insulin with fluorescein isothiocyanate. This method can determine variations in the concentrations to a precision of 0.5 logarithmic units. As much as 80 per cent of the fluorescent insulin could be immunologically altered without affecting the degree of inhibition of hemagglutination that a comparable amount of nonlabeled insulin would cause.

The biological activity of insulin was significantly decreased when conjugated with fluorescein isothiocyanate as measured by the one-hour blood glucose depression in rats. Whether the activity demonstrated with the fluorescent insulin was due to nonlabeled insulin or was a result of decreased activity of uniformly labeled insulin is unknown. An evaluation of each of the components of dialyzed fluorescent insulin may result in a more uniform, physiologically active, preparation which could be used for localizing the site of action of insulin in tissue.

SUMMARY

A fluorescein isothiocyanate derivative of insulin was prepared, and its biological, immunological, and physical properties were investigated.

Fluorescent insulin has at least two components by electrophoresis, each of which migrates faster than crystalline insulin.

The fluorescence of insulin labeled with fluorescein is augmented by serum proteins.

Insulin conjugated with fluorescein isothiocyanate reacts immunologically to a lesser degree than crystalline insulin when exposed to rabbit antibody against crystalline insulin as measured by hemagglutination. This altered activity could not be demonstrated in the cross inhibition of hemagglutination.

The biological activity of insulin in Wistar rats was significantly decreased when conjugated with fluorescein isothiocyanate.

SUMMARIO IN INTERLINGUA

Studios Relative al Proprietates Physic, Immunologic, e Biologic de Insulina

Esseva preparate un derivato de insulina a isothiocyanato fluoresceinic, e su proprietates biologic, immunologic, e physic esseva investigate.

Insulina fluorescente ha al minus duo componentes electrophoretic, e ambes migra plus velocemente que insulina crystallin.

Le fluorescentia de insulina marcate con fluoresceina es augmentate per proteinas seral.

Insulina conjugate con isothiocyanato de fluoresceina reage immunologicamente minus fortemente que insulina crystallin quando illo es exponite a anticorpore de conilio anti insulina crystallin, secundo masurationes del hemagglutination. Iste alterate activitate non poteva esser demonstrate in le inhibition cruciate de hemagglutination.

Le activitate biologic de insulina in rattos Wistar esseva reducite significativemente quando illo esseva conjugate con isothiocyanato de fluoresceina.

ACKNOWLEDGMENT

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Special Article

Diabetes in the Future

Elliott P. Joslin, M.D., Boston

Tennyson in *Locksley Hall,*

". . . dipt into the future far as human eye could see;
Saw the Vision of the world, and all the wonders that would be";

and it is as natural for us all to do the same even though our predictions will not be as perfect as his when he,

"Saw the heavens fill with commerce, argosies of magic sails,
Pilots of the purple twilight, dropping down with costly bales; . . ."

Indeed, Dr. Best only two years ago indulged in "Crystal Gazing: The Future in the Field of Diabetes."[1] In reading what he had to say, most of all I am impressed by the multitude of hints and opportunities for research which his paper contains. These actually run into the hundreds. The future is replete with problems and far more than we clinicians imagine. Even the incidence of diabetes is largely unknown. His ideas count far more than his prophecies, pleased as we are to learn what he predicts. It is a sobering thought to realize, as Dr. Best points out, that we have only to increase somewhat the severity of our tests for diabetes to discover that practically all of us are potentially diabetic. There is a limit to the quantity of sugar we can tolerate without its escape in the urine. Think of what this signifies for the prevention of diabetes and its prediabetic phase. Then he predicts insulin will be made synthetically; that an oral insulin is quite possible; and suggests how the absorption of the insulin molecule can be prolonged and also that a store of insulin which has accumulated in one part or another of the body may be drawn upon according to need. Especially does he urge research for the discovery of a stimulant to insulin formation. Attention is also called to the fact that many of the tissues may vary much in their need for it, just as now the brain utilizes carbohydrate without it. Dr. Best's comment on the pathology of the pancreas is noteworthy because a year or two ago one might have said that in 25 per cent of the cases of diabetes no histologic changes in the pancreas could be found; in another 25 per cent they were present and in 50 per cent indefinite. Today as a result of the work of Maclean and Ogilvie[2] and quite recently of others, especially Gepts[3] of Belgium, one feels assured that in all cases of diabetes, alterations in the islands of Langerhans exist and indeed there are hints not only that hyperplasia of these can take place but even regeneration, with all that implies. We certainly should give credit to Weichselbaum[4] and our own Dr. Cecil[5] who in 1909 and 1910 both reached much the same conclusion, although for years their work was passed over.

As for my own ideas upon diabetes in the future, they are more prosaic but they are facts I have been able to gather in a lifetime.

First of all, it is safe to say there are to be many more diabetics in the future than in the past simply because diabetics are living so much longer; indeed, between 1897 and 1914 my fatal cases had lived 4.9 years but at present the average for the group is eighteen years. Children with onset in the first decade of life survived 1.2 years but now twenty times as long, or twenty-six years, and by far the greater number of the children in our juvenile group are alive. Only last year I found that 131 of our patients had had diabetes over forty years. It was noteworthy in this group that there were more men than women, but this was partly to be explained because many of these were discovered by life insurance examinations, usually in an early stage and more males than females sought insurance. That group of patients always has lived longer than the average probably because its presence was discovered nearer its onset. Furthermore, there were many doctors and that has always been a cheering thought because it showed that a knowledge of the disease helped in extending longevity. The results are shown in table 1 and table 2. Here also in these tables are hints for the future because these 131 cases developed their diabetes two years before the discovery of insulin.

There will be more diabetics in the future because today so many diabetics conceive. Bouchardat,[6] the most noted diabetes clinician of all time, wrote in 1875 and again in 1883: "Women who have diabetes very rarely become pregnant. Among a very considerable number of diabetics who have consulted me, I do not remem-

TABLE 1

Duration of life subsequent to onset of diabetes among deceased patients in Naunyn era (1897-1914) and Best era (1956-1957)

	Naunyn era		Best era	
	Number of cases	Average duration	Number of cases	Average duration
	326	4.9 years	640	18.2 years
Ages				
0- 9	24	1.3	30	26.4
10-19	39	2.7	52	23.5
20-39	85	4.3	91	25.1
40-59	126	7.0	304	18.7
60 and over	51	4.4	159	10.2
Unknown	1	—	4	—

Tables 1 and 2 were prepared by the Statistical Bureau of the Metropolitan Life Insurance Company and aided by a grant from the Department of Health, Education, and Welfare, United States Public Health Service, National Institutes of Health.

TABLE 2

The changing average age at death and average duration of diabetes in two important eras of treatment

Era	Number of deaths	Average age at death, years	Average duration of diabetes, years
Naunyn:			
1897 to 5/31/14	326	44.5	4.9
Charles H. Best:			
1/1/50 to 12/31/55	4,376	64.7	15.6
1/1/56 to 12/11/57	640	64.7	18.2

ber to have seen a single pregnant woman." Today in our series we have approximately 2,000 pregnant women. Of this number, Dr. Priscilla White with her colleagues at the Faulkner and Boston Lying-In Hospitals have studied 1,225 meticulously. At first there were only about fifty-five mothers in 100 who delivered living children but now the number has risen to eighty-seven in 100. Abortions and miscarriages were common early in this century but today are rare. Indeed, this last year Dr. White tells me that during 1959 no patient was advised to have an abortion. Even those diabetics who formerly were considered in too serious condition to go on with their pregnancy now are allowed to do so. Class F, the severest group of her series of patients, once included 5 per cent of all the diabetic pregnancies but today it has doubled to about 10 per cent. In January 1960, one pregnant woman, thirty-one years old, with diabetes of twenty-nine years' duration, blind and with impaired kidneys, was delivered at the Boston Lying-In Hospital of healthy twins who are living today. She was carried to the thirty-fourth week of her pregnancy. Another woman who was also blind, in February 1960 also was delivered of a healthy baby. The pregnancy in each of these two cases did not appear to have any deleterious effect on the kidneys of the patient.

We are all learning much from the pregnant diabetic. Years ago it was noticed that the offspring of pregnant diabetic women were often large. Furthermore, it was observed that the babies of such women had large islands of Langerhans in which there was evidence of regeneration. The earliest case of a huge baby was that reported by Bennevitz[7] in 1824 in which was the sentence, "It appeared as if Hercules was the father." With ninety-five depancreatized male rats in Houssay's laboratory it was shown that injections of female hormones would delay the development of diabetes. Dr. White has observed that those of her cases who have taken hormones throughout the pregnancy require somewhat less insulin than before delivery and less than in the control group which took no hormones.

Realizing that diabetics today conceive and bear many children, the question arises whether they will pass on to their descendants a diabetic heredity. How important this is in a patient like Mrs. D., a diabetic who has fourteen living children and forty-five grandchildren. Think of the growing number of individuals with an hereditary condition, and with their increasing duration of diabetes, thus favoring their passing on the disease.

Frankly, I think all diabetes is hereditary. My reasons are: 1. That when I questioned each case of a hundred seeing me in succession at the office during 1959, I found that sixty patients knew of a diabetic heredity. These patients supposedly would live five or ten years or longer, and during that period thus furnish the possibility for a higher percentage of this manifestation of heredity. Among our Quarter Century Victory Medal diabetics with their diabetes now over thirty-four years' duration, the evidence of heredity of diabetes also amounts to more than 60 per cent.

With our series of identical twins, 48 per cent of the number developed diabetes in both twins whereas among the dissimilar twins only 3 per cent. Dr. White, in a study of the relatives of diabetic children, found five times as many cases of diabetes as were present in the relatives of a nondiabetic control population.

Inherited patterns take time to show themselves. Dr. N. developed his diabetes in 1900 and in 1908 I found no evidence of its inheritance in his family and indeed it did not appear through his lifetime ending in 1920. Last year, his son developed diabetes approximately sixty years after his father. When I read in a history which has obviously been taken quite casually,

"no diabetic heredity," I often think of Dr. N. and how after waiting nearly sixty years heredity was demonstrable.

Another factor responsible for the increase of the number of diabetics in the future is the aging of our population. Diabetes occurs most commonly between forty and sixty years of age. In 1870 the average age at death in the United States was twenty-five years but now is 61.8 years and the expectation of life is 69.8—reaching a peak in 1959. This is important because three fourths of the diabetics have their onset after forty years of age.

Women who have children were recognized long ago to have a greater chance of becoming diabetic than single women. Indeed the frequency of diabetes in married females has increased to twice that of single women between forty and sixty years of age. The incidence for single women is about the same as for males. Perhaps a part of the reason may be that married women weigh more than single women but that is not the whole explanation. New data are needed on this point.

Another observation made in the last year—although not directly bearing on this point—was that I ran across twenty-eight women who had given birth to one or more babies weighing twelve or more pounds. All of these women later developed diabetes. Among them, Mrs. B. had fourteen children, twelve of whom are now living. Her diabetes was discovered twenty-nine years after the birth of her twelve-pound baby. In four other mothers the interval was twenty-five to thirty-three years. The average number of children born to each of these mothers before they developed diabetes was five. Three had ten or more children and three had 13, 15 and 34 grandchildren respectively. The average duration of diabetes in the mothers was sixteen years and the average age at which it developed was forty-seven years. The weights of the mothers in this series were high, thirteen of the twenty-eight exceeding 200 pounds. Only one of the entire number was underweight. Inheritance of diabetes for twenty-four of the twenty-eight mothers in which it was known was 66 per cent.

Boulin had the right idea when he inaugurated his prediabetic clinic in Paris. To this came those who were overweight and were lacking in exercise—the cases described by Katsch as "civilization" diabetes; patients with marked heredity; those who were pregnant.

Overweight is the one factor which stands out so prominently in diabetics. When I studied 1,000 cases of diabetes and calculated their weights compared with standard weights and heights, I found that even with patients with onset in the third decade, only 10 per cent were ever underweight; in the fourth decade, 5 per cent, in the fifth decade, 3 per cent and in the sixth (between fifty-one and sixty years of age) of the 252 patients there was only 1 per cent whose weight was below standard. In one way or another this table is duplicated. Today we know that with the new calculations of the Insurance Actuarial Boards, that our population in general is approximately 15 to 20 pounds overweight. This applies not only to men but to women. The one factor which all doctors can emphasize in the prevention of diabetes is the avoidance of overweight, particularly in the families of diabetics during pregnancy and also in the Jewish population. It is striking today to see how this idea is spreading. How rare it is to find a young Negress who is overweight in contrast to a few years ago when statistics showed that Negro women in New York City had a higher percentage of diabetes than white women. It is fortunate that one is safe in urging reduction in weight because not only is it advantageous for diabetes but for vascular disease as well. A century ago, Bouchardat recognized this by telling his patients to earn their bread by the sweat of their brow. One patient between the ages of ninety and 100 who feared she would fall on the slippery streets in the winter, solved her problems by a stationary bicycle which she uses three times a day.

A revolution has taken place in the causes of death of diabetics. In the last century and even in large city hospitals in the first part of the present century, tuberculosis caused half of the deaths of diabetics. In the years 1897 to 1914, 64 per cent of our patients died of diabetic coma. Today tuberculosis in our group has practically disappeared (0.2 per cent), and death from coma among our patients has declined to 1 per cent although among hospital patients admitted in coma the mortality is nearer 3 per cent. Gangrene has fallen from 8 to 2 per cent and infections from 14 to 4 per cent. In contrast, disease of the blood vessels in the kidneys, heart and brain has risen from 18 per cent to 78 per cent. Renal disease has advanced from 3.4 to 11.3 per cent and cardiac diseases have risen from 6 per cent to 50 per cent; cerebral arteriosclerosis from 2.8 to 12.5 per cent. On reviewing the past and present it is certainly not logical to believe that the causes of death which are prevalent today will be the same in the year 2,000. Complications in the eyes are spectacular. Of the 874 individuals certified for blindness by the Commonwealth of Massachusetts between July 1,

1958, and June 30, 1959, diabetics numbered 148, exceeding all other groups. A hint that it can be avoided is shown by our ninety Quarter Century Victory Medal cases because in these patients, even after twenty-five years of diabetes, there was no involvement of the eyes when examined by specialists.

Our methods for the prevention and early discovery of diabetes in its preclinical stages should be greatly expanded. We should enlist all state medical societies, all doctors, all hospitals in the task. There should be a concentrated effort upon the relatives of known diabetics, particularly upon those above thirty years of age and especially upon all fat individuals. If each doctor would examine the urine of ten additional patients, and better still, secure analyses of the blood, consider how many new cases of diabetes would be found. Perhaps the most practical way would be to subsidize our private and public laboratories so that the tests would be free to all.

Having discovered the diabetic and prediabetic then one can treat and protect him. Today we know that control of the diabetes pays.

As yet no one has met our criteria for the Quarter Century Victory Medal whose diabetes was not controlled, particularly in its first year. These Medal cases were the reason for our Hospital Teaching Clinic where patients can be taught control of the disease and prevention of complications and by self-service at greatly reduced hospital expense.

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EDITORIAL

HYPOGLYCEMIC INSULIN REACTIONS WITHOUT WARNING SYMPTOMS

Hypoglycemic reactions due to the administration of insulin in diabetic patients, although usually harmless, may pose a major problem in patients who have unstable diabetes of long duration. The physician accustomed to combating hyperglycemia with keto-acidosis may well say, "A plague on both your houses," since unconsciousness occurs in this state as well as with hypoglycemia. However, the serious feature of hypoglycemic reactions recently stressed by Balodimos and Root* arises from the fact that these reactions sometimes occurred without the patient being aware of the usual warning symptoms. Patients of this group had learned to know the characteristic warning symptoms such as tremor, sweating, hunger or diplopia through many years of insulin administration. However, a stage was reached in which none of the usual symptoms was recognized. Patients continued to work automatically or became irrational, pugnacious, or even unconscious. These changes often occurred with striking suddenness. A series of sixty males and fifty-six females was reported among patients with long duration of diabetes treated with insulin. The average duration was eighteen years. The insulin dose varied from less than 20 units daily to 60 units. The series did not include patients who had reactions during sleep.

It did not seem that either the error of taking extra large doses of insulin or simple inattention to symptoms provided an explanation. The blood sugar levels were not strikingly different from those in other patients who had ample warning of approaching reaction.

Retrograde amnesia for periods varying from three minutes to one hour before the actual reaction was a feature in most of the patients. Electroencephalographic studies did not usually give any characteristic findings although some changes were observed in the tracings. The possibility that these reactions are actually epileptic

*Balodimos, M. C., and Root, H. F.: J.A.M.A. *171*:261-66, September 1959.

equivalents was thoroughly considered in consultation with various neurologists, but the use of anticonvulsive drugs did not result in any significant therapeutic benefit. Neuropathy affecting the autonomic or higher cerebral centers with actual impairment of the function of some neurons consequent upon repeated episodes of hypoglycemia might be a possible explanation.

Treatment and prevention included the division of the day's diet into five, six or seven meals. The taking of carbohydrate not only at the time of reaction but in shorter periods during the day proved of real benefit. The advantages and indeed the necessity of a constant rather than a fluctuating diet and the effect of exercise upon the action of insulin are most important points in the teaching of patients. Administration of Sorbitol, a polyalcohol, was tried with apparent benefit in older subjects but with less benefit in the younger ones. The replacement of a single dose, which includes long-acting insulin, by divided doses of insulin has often proved helpful. If a mixture of a long-acting insulin with Regular Insulin is taken before breakfast the number of units may be materially reduced by a second dose before the evening meal or at bedtime. Indeed in a few instances multiple small doses of Regular Insulin instead of the use of long-acting insulin have been effective.

It is important to instruct patients in the study of their individual symptoms. Thus, very mild, slight, warning symptoms such as lassitude, slight slowing in speech or thought, or headache must be emphasized to certain patients and accepted as warnings requiring the taking of carbohydrate. With further study of hypoglycemia and particularly further individual study in each case probably many such reactions can be prevented. The problem of these sudden reactions has serious implications, both legal and social, and is one to be approached by improvement in patient training and education and constant awareness on the part of the attending physicians.

HOWARD F. ROOT, M.D.
Boston, Massachusetts

BOOK REVIEWS

CLINICAL ENDOCRINOLOGY, I. *Edited by Edwin B. Astwood, M.D. $18.75, eighty-two contributors, sixty-eight chapters, 169 illus., pp. 744, Grune and Stratton, New York, 1960.*

This book comprises a series of chapters on recent developments and current concepts in selected topics of endocrinology, covering six major areas: I, Pituitary; II, Thyroid; III, Parathyroid; IV, Pancreas; V, Adrenal; VI, Reproductive System. The book also contains a section discussing miscellaneous topics of current interest such as: the effect of hormones on depot fat, obesity, hormones and the skin, hormone therapy of cancer, hormonal regulation of red cell production; and angiotension, a hormone of the kidney. The final section of the book is devoted to a timely and informative discussion of hormone assay technics and special tests.

The style, clarity and presentation, as well as the discussion of factual material, are uneven and in general tend to reflect the skill, experience, and depth of information of the large number of contributing authors. However, in spite of this criticism, most of the chapters are well written, concise, and well documented with generous use of tables, figures, diagrams and photographs. Furthermore, although the book is clinically oriented, a successful integration of the experimental with the clinical studies has been satisfactorily achieved.

One may indeed disagree with the editorial prejudice in the selection of topics and one may question the disproportionate allocation of space to some topics at the expense of others; but it is to the editor's credit that he has succeeded in covering in a single volume of this size such a wide area of topics of current interest and importance in endocrinology.

The title of the book is perhaps inappropriate in that it conveys the impression of being a textbook when in reality it is more in the nature of an extensive progress report on some of the newer developments and concepts in endocrinology. Hence, the book is not recommended as a basic source of information in endocrinology for either medical students or general physicians. Nevertheless, in bringing together the newer information on important current topics in a single volume, it should form a very useful adjunct and supplement to current textbooks in endocrinology. This is particularly true in respect to the section on hormone assays and special tests, which, in the opinion of this reviewer, is one of the best sections of the book.

THE CLINICAL SYNDROME OF DIABETES MELLITUS. *By John Lister. $4.50, pp. 234, 34 illustrations, Charles C Thomas, Springfield, Illinois, 1960.*

The author, known to readers of *The New England Journal of Medicine* for his "London Post" column, has attempted a monumental feat in compressing the subject of diabetes, its ramifications, treatment and philosophy, into twenty-one chapters totaling 234 pages. The result is a text too large for an outline and too small for a reference book, but as is stated in the preface, ". . . at present there seems to be a lack of a small textbook covering the essential clinical aspects of the disease."

The book attempts too much, with a resulting heterogeneous combination of detail and superficiality. The reader finds generous portions of diabetes history combined with a pinch

of recent research (Chapter 1), an excellent smattering of carbohydrate chemistry (Chapter 2), very thorough instructions for the use of electrolytes in the treatment of ketoacidosis (Chapter 6), detailed case histories of patients with hypoglycemia (Chapter 7), a 1½-page chapter (16) discussing prediabetes, and even a very commendable section covering the medical-social aspects of diabetes. The latter is a topic which many authors frequently sidestep or pretend does not exist. One wonders if the instructions concerning the use of Clinitest tablets and "How to Sterilize a Syringe by Boiling" (Chapter 8) might not better have been reserved for a patients' manual. While much of the material is well presented, the space devoted to the various topics would not always seem to correlate with the present-day relative importance of these subjects. A glaring weakness is the inadequacy of the section discussing oral hypoglycemic agents. Many of the cited observations have been superseded by recent knowledge. This, of course, is a problem common to all books in rapidly changing fields of medicine and in this case many recent significant facts have missed the publishers' deadline. Also, in a book obviously destined for distribution in this country, it would have been a simple matter for the publishers to add a page regarding activities of the American Diabetes Association as a supplement to the excellent material on The British Diabetic Association at the end of the book.

On the credit side, the book is informative, lucid, excellently illustrated, and easily read. The section on treatment, particularly the pages dealing with electrolyte imbalance, is especially good. While this is not a source book, it is fundamentally sound and should provide succinct reading for the student or practitioner who desires a view of the entire vista from one vantage point. Thus, while the author aimed at the planet Mars and missed, he did hit the moon, which is a very considerable achievement.

DIABETIC CARE IN PICTURES. *By H. Rosenthal, B.S., and J. Rosenthal, M.D. 3rd edition, $4.50, pp. 137, J. B. Lippincott Co., Philadelphia, 1960.*

This book is a conscientious attempt to present simplified statements with illustrations for the use of the patient. The fact that this is the third edition indicates that it has served a useful purpose.

As long ago as 1946 the Rosenthals, working at the Frances Stern Food Clinic in Boston, recognized the need for such a manual. This book has helped innumerable diabetics to carry out the orders of their physicians. There are many places in the United States and elsewhere that have neither food clinics nor classroom instruction for the diabetic. This book will enable patients in these areas to cope with the many details of proper regulation of their diabetes; it also will relieve physicians of this task and consequently save them considerable time.

The format is well arranged, and each of the nine chapters covers its subject in a conservative and succinct fashion. There are 137 illustrations to aid in the instruction of the patient. Some of these portray food models, syringes, types of insulin, technic of injection and tables of food values.

In passing, the reviewer wishes to remark that the quality of the paper and printing are excellent. He believes that this book will serve a very useful purpose and he heartily endorses it.

HOME GUIDE FOR THE DIABETIC. DIET INSTRUCTIONS, RECIPES AND OTHER GUIDANCE. *3s (42¢), pp. 30, Iliffe & Sons, Ltd., London, 1960.*

This brief, simplified guide for the diabetic patient was prepared from a handbook used in the Leicester Royal Infirmary Diabetic Clinic. It is somewhat similar to the handbooks prepared by the American Diabetes Association and certain pharmaceutical companies, although it goes into even less detail.

While the information contained is similar to that in the American publications, there are some exceptions. A single syringe is recommended, for example, for insulin preparations of varying concentrations. A greater proportion of the body surface is used for insulin injections than is customary in most American clinics.

A number of diabetic diet menus are provided, ranging from 1000 to 2500 calories, and lists are included of servings of carbohydrate- protein- and fat-containing foods, respectively, which may be used for exchanges.

Of interest is mention in the booklet of playing cards designed to familiarize patients with food exchanges. These are available, at least to British patients, from the Central Council of Health Education and The British Diabetic Association in London.

THE MECHANISM OF ACTION OF INSULIN. *Edited by F. G. Young. $7.50, pp. 320, Charles C Thomas, Springfield, Ill., 1960.*

This volume contains the collected papers and discussions from an international symposium held two years ago. The title might encourage the prospective reader to expect an explanation of how insulin acts in the living body. Having read it, he will realize that, thirty-seven years after the discovery of insulin, the thirty-seven authors and the forty-one other participants in the symposium are still far from sharing a unified picture of how insulin acts. He will find good evidence that insulin acts on a wide range of body tissues to change specific characteristics of the anabolism and catabolism of specific carbohydrates, fats, proteins and minerals in living animals, their excised tissues or in cell constituents from them.

He will find the field of research on insulin action described at a most interesting and stimulating stage, as when an appreciable number of genuine pieces of a puzzle are provided but are not yet fitted together. Possibly the integration of the insulin picture must await more quantitative information concerning the effects of insulin on the rates of transfer and transformation of body substances between and in specific body tissues. The action of insulin on the rates of glucose production by the liver, described by Tarding and Schambye at this symposium, represents an early step in this direction.

The faithful record of opinions and discussion between experts and the authors of the papers is a valuable feature of this symposium. Through it both the research specialist and the general practitioner can arrive at as integrated a picture of the state of knowledge of the action of insulin as was available at the time of the conference. The central position of insulin as a governor of many anabolic features of "energy metabolism" makes this collection of papers of interest to a wide range of biological scientists. This book on approaches toward the mechanism of insulin action is well worth reading.

ABSTRACTS

Allwood, M. J.: and Ginsburg, Jean (Sherrington Sch. of Physiology and St. Thomas's Hosp. Med. School, London S.E. 1, England): THE EFFECT OF INTRA-ARTERIAL ATROPINE ON BLOOD FLOW IN THE HAND AND FOREARM DURING INSULIN HYPOGLYCAEMIA. J. Physiol. *149*:486-93, December 1959.

Blood flow was measured in both hands and both forearms of normal human subjects by venous occlusion plethysmography before and after the administration of atropine into the brachial artery of one side. The atropine had no effect on the resting blood flow in the hand or in the forearm. Cholinergic vasomotor activity at rest is therefore absent in these areas.

During hypoglycemia following the intravenous injection of insulin (0.1 μ/kg. body weight), the blood flow increased in both forearms, but the increase was significantly less in the atropinized than in the control forearm. There was no significant difference between the increase in blood flow during hypoglycemia in the control and atropinized hands. G.A.W.

Antoniades, Harry N. (Protein Foundation Labs., Jamaica Plain, Mass.): STUDIES ON THE STATE OF INSULIN IN BLOOD: THE STATE AND TRANSPORT OF INSULIN IN BLOOD. Endocrinology *68*:7-16, January 1961.

Whole blood or serum is passed through a cationic exchange resin. Elution of the resin with acid, citrate, or ammonium hydroxide results in an eluate having insulin-like activity (rat adipose tissue and diaphragm assays). Exogenous crystalline insulin is not removed by the resin. Human blood insulin at body *p*H is a negatively charged molecule and would not be bound by the resin. These findings suggest that insulin is transported as a complex with basic protein and the latter by virtue of its positive charge adsorbs to the resin. The insulin-basic protein complex must be dissociated before full insulin activity can be noted by assay. The basic protein can be demonstrated by dialysis, centrifugation, and paper electrophoresis. Exogenous insulin added to serum does not bind to the basic protein. Similar complexes are found in the blood of diabetic patients and are distinct from antibody-insulin complexes.

H.L.W.

Antoniades, Harry N.: and Gundersen, Kare (Protein Foundation Labs., Jamaica Plain; Boston Dispensary & Dept. of Prev. Med., Tufts Sch. Med., Boston, Mass.): STUDIES ON THE STATE OF INSULIN IN BLOOD: DISSOCIATION OF PURIFIED HUMAN BLOOD INSULIN COMPLEX(ES) BY INCUBATION WITH ADIPOSE TISSUE EXTRACTS IN VITRO. Endocrinology *68*:36-42, January 1961.

Previous work has shown that most human insulin in blood exists in a complex with basic proteins. Insulin activity as measured by the rat diaphragm method is increased by dissociation of the complex. Insulin activity as measured by rat adipose tissue assay is not increased by previous efforts to dissociate the insulin-protein complex. An extract was prepared from adipose tissue which significantly increased the insulin-like activity of insulin-protein complex as measured by rat diaphragm. The active substance in adipose extract was not found in liver, kidney, or muscle. Electrophoresis of the adipose tissue extract showed that most activity resided in the α-globulin-albumin region. Adipose tissue may release free or biologically active insulin from its complex with basic protein. H.L.W.

Balodimos, Marios C.: and Kim, Hae Sok (Pipinou 21, Athens 8, Greece, and St. Vincent's Hosp., Jacksonville, Fla.): INSULIN REACTION AND RECOVERY IN DIABETIC PATIENT. J.A.M.A. *173*:1476-78, July 30, 1960.

A sixteen-year-old diabetic girl in diabetic acidosis received 1,800 units of Lente insulin over a twenty-hour period because the mother misunderstood telephone instructions and thought that "Regular Insulin" was the Lente insulin that her daughter was taking at the time. After unconsciousness of three hours' duration she was hospitalized and found to be in a hypoglycemic state. She was given continuous intravenous dextrose and tube feedings. She started responding after sixty hours of continuous treatment and for the next twenty-four hours was disoriented and complained of headache. Insulin therapy was resumed ninety hours after admission. A follow-up examination several weeks later did not reveal any neurological abnormality or mental deficiency. B.F.K.

Barnard, Donald M. (Dept. of Internal Med., Fargo Clinic, Fargo, N.D.): DIABETIC PREGNANCIES IN A COMMUNITY HOSPITAL. Journal-Lancet *80*:508-10, November 1960.

A series of forty-five pregnancies in thirty-one diabetic patients is reviewed. In ascertaining the extent to which diabetes increases the likelihood of an unsuccessful outcome, a pregnancy risk classification (proposed by White) is of considerable value. The general physical condition of the mother, duration and age of onset of diabetes are important determinants. Careful premedical and obstetric care improves the chance of a satisfactory pregnancy. H.L.W.

Bouman, P. R. (Dept. of Pharmacol., Univ. of Groningen, The Netherlands): RELEASE OF INSULIN FROM ISOLATED RAT PANCREAS IN VITRO. Acta endocrinol. *35*:560-67, December 1960.

In vitro experiments were performed to study the effect of rat pancreatic tissue upon the uptake of glucose by rat diaphragm. A solution in which normal pancreas had been incubated had no effect. However, the use of pancreatic tissue from rats in which the pancreatic duct had been ligated six weeks before the experiment caused an increased glucose uptake. When normal pancreatic tissue and diaphragm were incubated jointly, glucose uptake was increased. This did not occur with pancreas from alloxanized animals. Finally, the glucose uptake of the diaphragm was increased when it had been preincubated with the pancreatic tissue in a glucose-free medium prior to transfer to a glucose containing medium. These experiments were interpreted to demonstrate the release of insulin by rat pancreas in vitro.

In other experiments increased glucose concentrations or the presence of a sulfonylurea in the medium had a further

stimulatory effect on the release of the pancreatic insulin in vitro. S.B.B.

Bouman, P. R.; and Dermer, W. (Dept. of Pharmacol., Univ. of Groningen, The Netherlands): CARBOHYDRATE METABOLISM IN THE ISOLATED DIAPHRAGM OF ADRENALECTOMIZED RATS AS AFFECTED BY EXPOSURE TO ADRENALINE IN VIVO AND IN VITRO. Acta endocrinol. *35*:551-59, December 1960.

The glucose uptake and glycogen deposition rates of hemidiaphragm of adrenalectomized rats anesthetized with Nembutal were measured under the influence of epinephrin. This was studied with and without prior in vivo administration of epinephrin. The results indicated a three-phase action of epinephrin on carbohydrate metabolism of the muscle. This is discussed in detail. S.B.B.

Bouman, P. R.; and Dermer, W. (Dept. of Pharmacol., Univ. of Groningen, The Netherlands): EFFECTS OF ADRENALINE ON THE CARBOHYDRATE METABOLISM IN THE ISOLATED DIAPHRAGM OF INTACT AND ADRENALECTOMIZED RATS AS INFLUENCED BY "NEMBUTAL" ANAESTHESIA. Acta endocrinol. *35*:541-50, December 1960.

Hemidiaphragms of intact and adrenalectomized animals killed by decapitation were studied for the effect of the addition of epinephrin upon glucose uptake and glycogen deposition. This was studied with and without Nembutal anesthesia prior to decapitation. With anesthesia and adrenalectomy, glycogen deposition was decreased and glucose uptake only slightly decreased. This was considered secondary to high glycogen content due to prevention of epinephrin discharge by the anesthesia. This muscle preparation was considered most appropriate for the study of the effect of epinephrin upon carbohydrate metabolism of muscle. S.B.B.

Chernosky, Marvin E. (Dept. of Dermatology, Baylor Univ. Coll. of Med. & Hermann Hosp., Houston, Tex.): CURRENT CONCEPTS OF NECROBIOSIS LIPOIDICA. South. M. J. *54*:25-29, January 1961.

Necrobiosis lipoidica is a histologically and clinically distinct cutaneous entity. It is most frequently found on the anterior surface of the lower extremities in females and often presents a cosmetic problem because approximately one fourth of the lesions eventuate in ulcers. In contrast to the older view that these patients either had or would eventually develop diabetes mellitus, the author reports a statistical survey of 201 cases indicating that this is true in less than 50 per cent.

Similar histologic findings have been reported in patients with necrobiosis lipoidica regardless of the presence of associated diabetes mellitus. Further, the control of the diabetes mellitus does not cause disappearance of the skin lesions. The injection of hydrocortisone or prednisolone acetate into the lesion, preferably by use of the dermajector, causes complete disappearance of early lesions and improves to some degree the more advanced lesions. Apparently, also, it will stop the lesions from enlarging and progressing to ulceration. On the other hand, injection of heparin sodium solution into lesions has led only to slight reduction of the yellowish color, although histologic studies have shown a decrease in lipids in the treated tissue.

L.S.S.

Cornblath, Marvin (Sarah Morris Hosp., Michael Reese Med. Center, Chicago, Ill.): HYPOGLYCEMIA IN NEWBORN INFANTS. Illinois M. J. *118*:332-37, December 1960.

Eight infants with symptomatic hypoglycemia occurring on the second day of life are described. All were born to mothers who had toxemia with hypertension. Administration of glucose relieved signs and symptoms in these infants. Etiology of the hypoglycemia was obscure. All infants were premature by weight. Five of the eight recovered without sequelae after two weeks, two were mentally retarded, and one died at seventeen days of pseudomonas sepsis and toxoplasmosis. Perhaps some of the unfortunate sequelae seen in children of toxemic mothers may be due to unrecognized hypoglycemia. H.L.W.

Cox, George E.; and Taylor, C. Bruce (Dept. of Pathology, Evanston Hosp. Assoc., Evanston, Ill.): CHOLESTEROL SYNTHESIS, DIET, AND SPECIES. Illinois M. J. *118*:282-83, November 1960.

Data are summarized which support the conclusion that the human liver supplies only a small part of plasma cholesterol in contrast to the dog and the rat. Extrahepatic tissues provide the major amount of serum cholesterol with the diet contributing one fourth or one third. If cholesterol synthesis inhibitors are to be successful in man they will have to act on extrahepatic tissues. H.L.W.

Craig, James W.; Miller, Max; Mills, Frank D.; and Nickerson, Naomi (Sch. of Med., Western Reserve University, Cleveland, Ohio): COMPARISON OF ACUTE HYPOGLYCEMIC POTENCIES OF TOLBUTAMIDE AND CHLORPROPAMIDE. J.A.M.A. *172*:779-82, Feb. 20, 1960.

Each of twenty-one diabetic patients was tested with tolbutamide and chlorpropamide administered intravenously. Blood glucose concentration and plasma drug levels were measured after the administration of the drug. At all four dose levels of the sodium salts of the drugs (1, ½, ¼ and ⅛ gm.), similar plasma drug levels and a similar lowering of the blood glucose concentration were produced by the two drugs. It is concluded that the two compounds probably do not differ in their intrinsic hypoglycemic potencies. The lower dosage requirement of chlorpropamide as compared to tolbutamide is due to the higher blood drug levels maintained by a given dosage schedule of chlorpropamide. B.F.K.

Culver, Gordon J.; and Tannenbaus, Joseph (Univ. of Buffalo, Sch. of Med., and the Buffalo Gen. Hosp., Buffalo, N. Y.): CALCIFICATION OF THE VAS DEFERENS IN DIABETES. J.A.M.A. *173*:648-51, June 11, 1960.

One hundred diabetics selected at random were studied for radiographically demonstrable calcification in the vas deferens. This abnormality was found to be present in eleven patients in the fourth, fifth and sixth decades. There was no correlation between the duration or severity of diabetes and the degree of vas deferens calcification, nor was there any correlation between the degree of calcification of the vas deferens and calcification of the pelvic arteries. About 70 per cent of men with calcification of the vas deferens can be expected to have diabetes. B.F.K.

DiGeorge, Angelo M.; and Auerbach, Victor H. (Depts. of Pediatrics & Physiological Chemistry, Temple Univ. School of Med., & St. Christopher's Hosp. for Children, Philadelphia, Pa.): LEUCINE-INDUCED HYPOGLYCEMIA: A REVIEW AND SPECULATIONS. Am. J. M. Sc. *240*:792-801, December 1960.

The authors thoroughly review leucine-induced hypoglycemia of infancy, its relative incidence as the etiologic agent in hypoglycemia, its familial occurrence, its associated histopathology in the pancreas, its etiology and mechanism, and its treatment and prognosis.

The suspicion that McQuarrie's idiopathic hypoglycemia of infancy represented a group of etiologically unrelated entities was confirmed by Cochrane's demonstration in 1955 that L-leucine, or L-leucine-rich casein, provoked hypoglycemia in certain but not in all such infants. Based upon the oral administration of 150 mg./kg. of body weight (or 1.5 gm. of casein/kg.), Cochrane reported positive reactions in 60 per cent of infants with idiopathic hypoglycemia. Other authors have cited varying figures, suggesting that one third of such infants are leucine-sensitive and that the entity is not excessively rare. The accepted familial occurrence of idiopathic hypoglycemia apparently is applicable to the leucine-sensitive category in the limited number of cases studied. Investigation of the hypoglycemic effect of valine and of all eighteen naturally occurring amino acids and related metabolic products has disclosed only leucine and alpha-ketoisocaproic acid unequivocally to exert such action, possibly from the transamination in vivo of alpha-ketoisocaproic acid to leucine.

Study of plasma-insulin activity after leucine production of hypoglycemia revealed increased activity both when determined by the rat hemidiaphragm method and by specific immunoassay of endogenous plasma insulin. In certain instances, analyses upon aliquots by both methods gave confirmatory results.

Histopathologic findings in cases of idiopathic hypoglycemia have disclosed no constant histologic pattern and have not yet revealed the validity of the seemingly high proportion of islet tissue hyperplasia observed in leucine-sensitive children. This view is supported by the leucine production of hypoglycemia and of increased serum insulin levels in cases of insulin-secreting pancreatic tumors and by the lost sensitivity to leucine following extirpation of the tumors.

Presumably leucine brings about increased production or release of insulin or both by an unknown mechanism, just as the basis for the same action by glucose is unknown. This hypothecates a normal relationship between leucine and insulin release, with aberration of the normal control mechanism in cases of leucine-sensitive hypoglycemia. This concept obtains support from the report in 1960 by the authors that leucine but not glycine produced a small but statistically significant depression of blood sugar level in eighteen normal children.

The dominant consideration in therapy is avoidance of hypoglycemic cerebral damage, especially in infancy. It is recommended that diets low in leucine be administered by use of human rather than cow's milk because the former contains substantially more leucine. The diet must provide sufficient leucine to meet its minimal daily requirement of 150 to 230 mg./kg. as an essential amino acid. Postprandial hypoglycemia may be forestalled by furnishing 1 to 2 oz. of fruit juice thirty minutes after feeding. Corticosteroid therapy previously advised for idiopathic hypoglycemia has been reported upon unfavorably in the leucine-sensitive variety of the disorder, possibly because the steroid increases the level of free circulating leucine as part of its gluconeogenetic action. The gravity of the therapeutic problem is illustrated by Cochrane's experience that six of eight leucine-sensitive hypoglycemic infants remained mentally retarded, despite the treatment of all by the sixth month of life. L.S.S.

Driscoll, Shirley G.; Benirschke, Kurt; and Curtis, George W. (Depts. of Pathology and Obstetrics & Gynecology, Harvard Medical School, Boston Lying-in Hosp., Boston, Mass.): NEONATAL DEATHS AMONG INFANTS OF DIABETIC MOTHERS: POSTMORTEM FINDINGS IN NINETY-FIVE INFANTS. Am. J. Dis. Child. *100*:818-35, December 1960.

Necropsy findings in ninety-five young infants of diabetic mothers are reviewed. Fetal gigantism and generalized visceral enlargement were infrequent, but there was a tendency to cardiomegaly and decreased brain weight. Hypertrophy and hyperplasia of the islets of Langerhans occurred in 81 per cent of the cases. This seemed to be related to relative body weight. The hyaline membrane syndrome accounted for 52 per cent of the deaths. Fatal congenital malformations were found in 17 per cent. The findings are discussed in relation to possible causes. R.L.J.

Edelman, Jerry; Kershbaum, Alfred; Sandberg, Herschel; and Bellet, Samuel (Div. of Cardiology, Philadelphia Gen. Hosp., Philadelphia, Pa.): THE EFFECT OF PROBENECID ADMINISTRATION ON BLOOD LIPIDS AND LIPID TOLERANCE CURVES IN SUBJECTS WITH MYOCARDIAL INFARCTION. Am. J. M. Sc. *241*: 96-102, January 1961.

Probenecid, as a uricosuric agent, was given to ten patients with myocardial infarction, four of whom had hyperuricemia, to determine whether the lipid patterns in these cases would change as the uric acid levels fell. A definite decline of serum uric acid was obtained in each, but this was not associated with any significant group alteration of lipid patterns in short term experiments of two or three weeks. L.S.S.

Field, James B.; Pastan, Ira; Herring, Betty; and Johnson, Phyllis (National Inst. of Arthritis and Metabolic Dis., National Inst. of Health, Bethesda, Md.): STUDIES OF PATHWAYS OF GLUCOSE METABOLISM OF ENDOCRINE TISSUES. Endocrinology 67:801-06, December 1960.

An active hexose monophosphate shunt has been shown in adrenal, ovary, testis, pituitary and parathyroid glands. While TSH stimulates glucose oxidation in the thyroid, similar results could not be demonstrated in other endocrine glands using appropriate trophic hormones.

It has been suggested elsewhere that ACTH affects adrenal steroid production through 3'5' cyclic adenosine monophosphate (AMP) which promotes glycogenolysis. In this view it would be expected that added ACTH or 3'5' cyclic AMP would interfere with the oxidation of added labeled glucose since they preferentially induce utilization of glucose-6-phosphate from glycogen. Added ACTH or AMP did not, however, decrease utilization of added labeled glucose raising question about the mechanism of ACTH action.

The hexose monophosphate shunt provides reduced TPN which may be an important factor in the production of hormones. H.L.W.

Fischer, Alfred E.; and Moloshok, Ralph E. (Dept. of Pediatrics, Mount Sinai Hosp., New York, N. Y.): DIABETIC AND PREDIABETIC PREGNANCIES WITH SPECIAL REFERENCE TO THE NEWBORN. J. Pediat. 57:704, November 1960.

Studies were carried out on infants born to ninety-seven women with clinical diabetes and sixty-two with decreased tolerance to glucose. The data are analyzed to determine total fetal loss, loss of viable births, and neonatal mortality. It is suggested that careful medical and obstetrical attention from the earliest weeks of the pregnancy exerts a favorable influence on fetal survival. Evidence is presented to show that the mortality figures for infants born to prediabetic mothers within five years of the onset of the disease are similar to those for

infants of diabetic mothers, but this is not true for infants born more than five years before the onset of diabetes.

Management of the delivery and of the infant are discussed briefly, including the indications for and timing of obstetrical intervention in the pregnancy. R.L.J.

Folkart, Gladys R.; Dancis, Joseph; and Money, William L. (Dept. of Pediat. and Dept. of Obstetrics and Gynecology, New York University - Bellevue Med. Center and the Sloan-Kettering Inst. for Cancer Res., New York, N. Y.): TRANSFER OF CARBOHYDRATES ACROSS GUINEA PIG PLACENTA. Am. J. Obst. & Gynec. *80*:221-22, August 1960.

A technic is described for perfusing the intact guinea pig placenta in utero and making measurements of rates of transfer of various substances in both directions. The technic is relatively simple and should be a valuable research tool. Some of the data reported concern the rates of transfer of various carbohydrates across the placenta. Stereo-isomerism was found to affect the rates of transfer of pentoses, D-xylose being transferred at a faster rate than L-xylose. Among the hexoses fructose was transferred slowly and glucuronic acid not at all. No difference was shown in the transfer rates of alpha and beta glucose. R.L.J.

Goetz, Frederick C. (Univ. of Minnesota Med. Sch., Minneapolis, Minn.): WHY DETECT DIABETES? Postgrad. Med. *28*:A-24,26,28, December 1960.

The author discusses the basic aims of diabetes detection campaigns and the results to be expected. The cost of discovering one new case is from $15 to $75. The intangible benefits of such a campaign may outweigh that of actual case finding. S.B.B.

Gold, Solomon (205 Mount Royal West, Montreal, Que., Canada): LIPODYSTROPHY RESULTING FROM INSULIN INJECTIONS: TREATMENT BY COCONUT OIL INJECTIONS. Canad. M.A.J. *83*:1104, Nov. 19, 1960.

The author reports the results of the use of coconut oil in the therapy of insulin lipodystrophy in three adult diabetics. The coconut oil was sterilized by boiling and injected by a twenty-gauge needle cooled to skin temperature. The dose is that required to make the depressed area level with the skin. Because of slight return of the depression of the skin, this was repeated once in each patient after an interval of four to six weeks. The improvement persisted in all patients. S.B.B.

Goodner, Charles J.; and Freinkel, Norbert (Harvard Med. School, Boston, Mass.): CARBOHYDRATE METABOLISM IN PREGNANCY: THE TURNOVER OF I^{131}-INSULIN IN THE PREGNANT RAT. Endocrinology *67*:862-72, December 1960.

The disappearance of radio-iodinated insulin from the blood of normal pregnant and postpartum rats was studied. Pregnancy significantly decreases the half time for the disappearance of insulin as compared to the normal. Postpartum animals showed a prompt return to the normal half time. Insulin metabolism is altered by pregnancy and may account for the increased need for insulin often seen in that circumstance. H.L.W.

Hamwi, George J.; and Skillman, Thomas G. (Ohio State Univ. Health Center, Columbus, Ohio): THE CLINICAL USEFULNESS OF ORALLY ADMINISTERED HYPOGLYCEMIC AGENTS. Postgrad. Med. *27*:687-95, June 1960.

Tolbutamide appears to be effective in older patients with mild diabetes; chlorpropamide seems the treatment of choice in more severe cases. Phenformin may be used alone or as an adjunct to insulin therapy. Generally, side effects are sufficiently mild to be counteracted by cessation or reduced dosage of the drugs. In some cases treated with carbutamide and metahexamide, severe damage has occurred. W.R.K.

Healy, M. F.; and Arneaud, J. D. (Gen. Hosp., Port of Spain, Trinidad, B.W.I.): PRELIMINARY OBSERVATIONS ON MANAGEMENT OF DIABETES WITH "STABINOL." Brit. M.J. *2*:913-15, Sept. 24, 1960.

One of the most potent of the substituted thiadiazoles, the methoxy isobutyl derivative known as FWH 114, or "stabinol," was made available for this study. The hypoglycemic effect of stabinol has been evaluated on a group of thirty-three patients with diabetes of the maturity onset type. Of this group three were lost to the study. Of the remaining thirty patients, fifteen (50 per cent) were better controlled with the oral agent than they had previously been on insulin therapy, nine (30 per cent) were controlled equally well on either regimen, and six (20 per cent) were not as well controlled on oral therapy as they were on insulin therapy. Liver function studies and blood and urine findings remained normal throughout the study. There were no side effects or abnormal reactions. W.R.K.

Heller, Irving H.; and Hess, Sigurd (Donner Lab. of Experimental Neurochemistry, Montreal Neurological Inst., McGill Univ., Montreal, Que., Canada): ACTION OF INSULIN ON THE RESPIRATION OF RAT SCIATIC NERVE. Lancet *2*:406-07, Aug. 20, 1960.

Insulin, added in vitro, is capable of stimulating respiration in the resting nerve, probably by enhancing the utilization of glucose, endogenous or exogenous. Insulin added in vitro cannot stimulate the respiratory rate of nerve obtained from rats in hypoglycemic coma, probably because the endogenous glucose supply is depleted in these nerves. Glucose given intraperitoneally stimulates respiration in the resting nerve, presumably by increasing the amount of endogenous glucose in the nerve. It appears likely that the effect of insulin is due to some action on surface membranes of the nerve, permitting further entry of glucose. W.R.K.

Hellerström, Claes; and Hellman, Bo (Histological Dept., Univ. of Upsala, Sweden): SOME ASPECTS OF SILVER IMPREGNATION OF THE ISLETS OF LANGERHANS IN THE RAT. Acta endocrinol. *35*:518-32, December 1960.

A modification of Davenport's alcoholic silver stain was used to study the normal rat pancreas which had been fixed in formalin or Bouin's solution. This stain delineated only a portion of the A cells. The stain was studied in detail, and some postmortem change was found to be essential to its action. Varying the staining conditions did not result in the staining of all the A cells. The authors concluded that A cells may be classified into two types by this technic. S.B.B.

Henneman, Dorothy H.; and Vandam, Leroy D. (Div. of Anesthesia, Dept. of Surgery, Peter Bent Brigham Hosp., and Harvard Medical Sch., Boston, Mass.): EFFECT OF EPINEPHRINE, INSULIN, AND TOLBUTAMIDE ON CARBOHYDRATE METABOLISM DURING ETHER ANESTHESIA. Clin. Pharmacol. & Therap. *1*:694-702, November-December 1960.

The administration of epinephrin during ether anesthesia in man produced a greater than normal rise in blood glucose and elevations in pyruvate, lactate, and citrate levels less than

would be expected from a combined effect of ether and epinephrin. Epinephrin failed to depress serum inorganic phosphorus levels or to prevent the progressive rise in levels observed during ether anesthesia. The administration of insulin during ether anesthesia failed to depress blood glucose and inorganic phosphorus levels. As with epinephrin, the changes in levels of pyruvate, lactate, and citrate were less than those expected from the combined effects of ether and insulin. Increased sensitivity to insulin was observed during thiopental anesthesia. The data suggest that ether may alter the cellular transfer and phosphorylation of glucose in a manner not fully explained by the reflex release of endogenous epinephrin. The administration of tolbutamide during ether anesthesia depressed both blood glucose and serum inorganic phosphorus levels and prevented the progressive rise in glucose normally observed during ether anesthesia. The effect of tolbutamide administered during ether anesthesia suggests that it may have a glycostatic effect independent of its effect on insulin secretion. W.R.K.

Hennigar, Gordon R.; Cohen, Richard J.; and Katz, Harvey P. (Dept. of Pathology, State Univ. of New York Coll. of Medicine, Downstate Med. Center, Kings Cty. Hosp., Brooklyn, N.Y.): NODULAR GLOMERULOSCLEROSIS: CLINICO-PATHOLOGICAL CORRELATION OF 40 ADVANCED CASES. Am. J. M. Sc. *241*:89-95, January 1961.

Because of controversy with respect to the prior occurrence of clinical acidosis and to the existence of diminished insulin requirement on the part of patients with advanced manifestations, the authors critically reviewed the forty cases of advanced nodular glomerulosclerosis detected in 525 necropsies performed upon diabetics from 1950 through 1959.

In this series of forty cases of Grade III and IV (by Epstein and Zupa's classification) diabetic nodular glomerulosclerosis, nineteen patients had a history of diabetic acidosis. At no time was there evidence of a diminished insulin requirement in these patients. Hypertension was present in 85 per cent of the cases. Marked proteinuria was consistently found in all of the patients.

Pathologic changes in the islets could not be correlated with those seen in the glomeruli, nor were they related to the presence or absence of diabetic acidosis. Of the nineteen patients with diabetic acidosis and nodular glomerulosclerosis, fifteen showed no lesions in the islet cells of the pancreas. Of the remaining twenty-one patients without evidence of diabetic acidosis, twelve showed islets free of pathologic changes.

The histological identification of nodular glomerulosclerosis requires strict criteria as laid down by Kimmelstiel and Wilson. Both hyaline sudanophilic nodules and advanced diffuse intercapillary glomerulosclerosis with prominent intercapillary material have been incorrectly referred to as the lesion of nodular glomerulosclerosis. L.S.S.

Houssay, B. A.; and Penhos, J. C. (Inst. of Biology and Experimental Medicine, Buenos Aires, Argentina): PANCREATIC DIABETES AND HYPOPHYSECTOMY IN THE SNAKE XENODON MERREMII. Acta endocrinol. *35*:313-23, November 1960.

The authors studied diabetes produced by pancreatectomy in 290 snakes of the species Xenodon Merremii. As in lizards and ducks, the immediate effect of pancreatectomy was hypoglycemia. This was followed after three or more days by normal, then elevated blood sugar levels. Hypophysectomy before or after pancreatectomy ameliorated the hyperglycemia.

Alloxan produced a transient blood sugar elevation, followed by hypoglycemia but not diabetes.

Sulfonylurea drugs had hypoglycemic effects in the normal but not in the pancreatectomized snakes. The actions of glucagon, insulin, cortisol and somatotrophin were all observed. The latter produced a rise of blood sugar only in pancreatectomized animals. S.B.B.

Jones, George M. (Dept. of Int. Med., Univ. of Texas Southwestern Medical Sch., Dallas, Tex.): POSTHYPOGLYCEMIC ENCEPHALOPATHY. South. M.J. *53*:1258-62, October 1960.

Numerous cases of changes in personality and mental and neurologic abnormalities following hypoglycemia and persisting after the levels of blood sugar have become normal have been reported previously. The physiologic and pathologic changes responsible for the clinical behavior are briefly discussed. It is recommended that the term posthypoglycemic encephalopathy be used for this definite clinical entity. Physicians should be aware of this very serious complication of hypoglycemia and combat any prolonged low blood sugar level vigorously. W.R.K.

Kennan, Alfred L.; and Proper, George (Depts. of Gynecology and Obstetrics, University of Wisconsin School of Medicine, Madison, Wis.): BLOOD AMMONIA IN RATS GIVEN ALLOXAN. Am. J. M. Sc. *240*:776-81, December 1960.

The authors report the blood ammonia to be elevated in rats given a large dose of alloxan to produce diabetic coma. The basis for this elevation in blood ammonia is considered and attributed to the combined deleterious effect of the alloxan upon the pancreas, liver and renal tubules. L.S.S.

Kirschner, Robert; and Leopold, Irving H. (Grad. Sch. of Med., Univ. of Pennsylvania, and Res. Dept., Wills Eye Hosp., Philadelphia, Pa.): RETINAL CHANGES IN THE ALLOXAN DIABETIC RAT MAINTAINED ON A HIGH FAT DIET: PRELIMINARY STUDIES UTILIZING A NEW FLAT RETINA TECHNIQUE. Arch. Ophth. *64*:681-84, November 1960.

In a pilot study using twelve alloxan diabetic Wistar rats, no aneurysmal retinopathy was noted after three months. Four of six alloxanized animals receiving a high fat diet showed changes interpreted as segmental thinning of the capillary reticulum. The implications are discussed. W.R.K.

Krecke, H. J.; Linke, A.; and Mueller, W. (Dept. of Medicine, Univ. of Heidelberg, Germany): SPONTANEOUS HYPOGLYCEMIA IN PRIMARY CARCINOMA OF THE LIVER. Deutsches Arch. klein. Med. *206*:102-18, 1959.

A seventy-one-year-old man with a history of alcoholism was hospitalized because of a severe hypoglycemic reaction. The diagnosis of cirrhosis with primary carcinoma of the liver was suggested by the clinical finding of a markedly enlarged, nodular liver, and confirmed by laparoscopy and liver biopsy and, finally, by the postmortem examination.

The patient was not jaundiced and not cachectic. The final illness lasted about two months and was characterized by hypoglycemic reactions, recurrent almost daily; these were most difficult to combat and occurred in spite of a daily intake of 1,200 gm. of carbohydrate. The adrenalin test showed a moderate hyperglycemic response; steroid administration was ineffective. The patient died in hypoglycemia. The histological examination of the pancreas failed to show islet cell hyperplasia or an islet cell tumor. Its insulin content was found to be less than normal. The liver was transformed into a

large tumor mass, practically free of glycogen. The tumor extract did not have any hypoglycemic activity.

The authors speculate that the hypoglycemic syndrome was due to an excessive glucose-consumption of the tumor cells, which may have deprived the organism of the available glucose-6-phosphatase (German). M.G.G.

Lakin, Mervyn; Zeytinoglu, Ibrahim; Younger, Mary D.; and White, Priscilla (Joslin Clin., Boston, Mass.): EFFECT OF CHLOROTHIAZIDE ON INSULIN REQUIREMENTS OF PREGNANT DIABETIC WOMEN. J.A.M.A. *173*:353-59, May 28, 1960.

Chlorothiazide was used in the treatment of sixty-one female diabetics throughout the course of their pregnancy. The average case was seen initially in the ninth week and then weekly throughout the course of pregnancy. In about half of the patients the average daily dose of 500 mg. was increased to 1,000 mg. after the twenty-eighth week of pregnancy. The drug had no effect on the insulin requirement during pregnancy and there appeared to be no long-term effect as measured by the prepartum and postpartum insulin requirements. The patients were not given potassium supplements and the serum potassium level was not affected. This was thought to be due to the fact that the patients were on a 2-gm. sodium diet, high in protein and fruits, with an average potassium intake of 4,500 mg. daily as compared to 3,700 mg. in the prepregnancy state. B.F.K.

Landau, Bernard R. (Sch. of Med., Western Reserve Univ., Cleveland, Ohio): GLUCONEOGENESIS AND PYRUVATE METABOLISM IN RAT KIDNEY, IN VITRO. Endocrinology 67:744-51, December 1960.

Gluconeogenesis from pyruvate occurs in kidney slices from normal, alloxan-diabetic and cortisone-treated rats and is greater than normal in the last two groups. The cortisone-treated rats showed a greater than normal incorporation of the C-14 label from pyruvate in glycogen. Diabetes mellitus and cortisone administration alter the metabolism of pyruvate by the kidney. H.L.W.

Levin, Noah B.; and Kohn, Kate H. (Mt. Sinai Hosp., Chicago Med. Sch. & Rest Haven Rehabilitation Hosp., Chicago, Ill.): POSTPRANDIAL HYPERGLYCEMIA IN THE DIAGNOSIS OF DIABETES MELLITUS. Illinois M. J. *118*:329-31, December 1960.

The fasting and one-hour postprandial blood sugars were studied in 241 elderly patients to determine the efficacy of the latter determination as a diagnostic measure. Fasting blood sugar was high in five patients and they were eliminated from study. Two hundred and thirty-six patients had a fasting blood sugar less than 110 mg. per 100 ml. and 30 per cent of these had a one-hour value over 150 mg. per 100 ml. This percentage was considered too high and it was concluded that the one-hour postprandial blood sugar was an unreliable diagnostic tool. H.L.W.

McCullough, Campbell C., Jr. (Kansas City, Mo.): DIABETIC NEUROTROPHIC ULCERS OF THE FEET: SURGICAL CORRECTION BY WEDGE OR PIE-RESECTION. Missouri Med. 58:117-19, February 1961.

The author describes a technic for surgical treatment of neurotrophic ulcers of the foot in diabetes by which a limited excision is carried out, avoiding the necessity of amputation. The procedure consists of removal of the head of the metatarsal bone underlying the ulcer, along with the necrotic tissue present. The foot remains useful and prolonged immobilization is avoided. Four case histories are presented. R.L.J.

McLendon, Harold; and Bottomy, John R. (Dept. of Obstetrics and Gynecology, Emery Univ., Atlanta, Ga.): A CRITICAL ANALYSIS OF THE MANAGEMENT OF PREGNANCY IN DIABETIC WOMEN. Am. J. Obst. & Gynec. 80:641-53, October 1960.

One hundred and seventy pregnancies occurring in 106 diabetic patients over a nine-year period were studied. The total fetal survival rate was 66.6 per cent, and the viable fetal salvage rate, excluding abortions and previable births, was 76 per cent. Abortion, prematurity, and malformations were only slightly more common than in the total clinic group. Acidosis was present in 16.2 per cent of the cases in which intrauterine death occurred, and the fetal survival rate in those showing acidosis was 53.8 per cent. Better prenatal care and early delivery reduced the toll from toxemia and essential hypertension. Stilbestrol in fifty cases did not reduce the incidence of acidosis and toxemia. Better prenatal care, proper timing, and mode of delivery seem to offer the best means of improving fetal survival rate. R.L.J.

Meyer-Hofmann, G.; Schwarzkopf, H.; and Hartmann, H. (Dept. of Medicine and Pathology, Municipal Hospital, Hanover, Germany): SPONTANEOUS HYPOGLYCEMIAS IN EXTRAPANCREATIC TUMORS. Deutsche med. Wchnschr. 85:2106-12, Nov. 25, 1960.

After a review of the relatively small number of twenty-seven cases of this syndrome, reported in the world literature, the authors describe their observations in a thirty-five-year-old female with a metastasizing dysgerminoma of the ovary. The primary tumor as well as the metastases were relatively small. The latter involved the vagina, the cul-de-sac, the lungs and the tail of the pancreas.

In addition to the severe and recurrent hypoglycemic attacks, the patient developed endocrinological changes: acromegalic features, a nodular struma, hyperplasia of the eosinophil and basophil cells in the pituitary, hypertrophy of the adrenals and glandular hyperplasia of both mammae. In spite of continuous glucose infusions, the blood sugar level was seldom higher than 80 mg. per 100 ml. and usually fluctuated between 50 and 60 mg. per 100 ml., with occasional levels as low as 25 mg. per 100 ml. An attempt to abolish the hypoglycemia by alloxan administration was unsuccessful, even with intravenous administration of a total of 1,025 mg./kg. of alloxan.

At the postmortem examination, no toxic damage due to alloxan was found in the liver or in the kidneys. The pancreatic islets showed a normal alpha-beta cell ratio with no evidence of beta cell necrosis or of islet cell hyperplasia. The authors suggest that the hypoglycemia was due to insulin or an insulin-like substance produced by the tumor. The accompanying endocrine changes are interpreted as evidence of attempts of the organism to counteract the hypoglycemia by overactivity of contraregulatory hormones of the pituitary, the thyroid and the adrenals (German). M.G.G.

Moore, Clemmie Everly; Kay, Jacob L.; Desmond, Murdina M.; and Dutton, Robert V. (Dept. of Pediatrics, Baylor University College of Medicine, Houston, Tex.): TRANSITIONAL DISTRESS IN INFANTS OF DIABETIC MOTHERS. J. Pediat. 57:824, December 1960.

Reference is made to a previous article describing a group of symptoms in newborn infants, referred to as transitional distress, occurring in infants whose umbilical cords continued

to pulsate for some time after birth. The finding that some of the infants were born to diabetic mothers led the authors to observe a group of infants of diabetic mothers for evidence of transitional distress as described in the paper. The group of symptoms is similar to those seen in older individuals recovering from anoxic or anesthetic depressions, suggesting a possible etiology. Twenty-eight infants were observed. Seven had no distress, five showed transient symptomatology without any definite clinical pattern, and sixteen showed transitional distress. The severity of maternal diabetes appeared to be the most important factor related to the presence of distress in the infant. Two infants died in the early neonatal period. Five of the survivors were considered to be neurologically abnormal on follow-up examination (nine to twenty-two months). R.L.J.

Pell, Sidney; and D'Alonzo, C. A. (E. I. du Pont de Nemours and Company, Inc., Wilmington, Del.): DIABETES MELLITUS IN AN EMPLOYED POPULATION. J.A.M.A. *172*:1000-06, March 5, 1960.

A survey of diabetics employed by a large company was made over a twelve-month period in 1956 and 1957. The company does not, as a matter of policy, refuse employment to anyone because of diabetes mellitus, except when the applicant cannot demonstrate that he can control the disease. Among 90,596 employees there were 408 diabetics, a rate of 4.5 per thousand. Among salaried employees, prevalence was inversely related to the job level. The disease was less prevalent among production workers than among the lower level salaried employees. No significant difference was found in the distribution of ABO blood groups between the diabetics and the controls. Although obesity was more common among the diabetics, 92.1 per cent of them had been able to lose weight since the discovery of their disease. Hypertension was more common among the diabetics than the controls, but overweight could not be singled out as the main cause. A history of peptic ulcer was more common among the controls than the diabetics. B.F.K.

Perkin, Frank S. (Wayne State Univ., College of Med., and Harper Hosp., Detroit, Mich.): PRACTICAL USE OF HYPOGLYCEMIC AGENTS. J.A.M.A. *173*:36-37, May 7, 1960.

The use of diet, insulins, sulfonylureas and phenformin alone and in combination is discussed. Phenformin has replaced as much as 70 units of insulin daily in one instance. It is increasingly necessary to evaluate each diabetic patient so that the proper hypoglycemic agent or combination of agents may be used. B.F.K.

Petersson, Birger; Hellerström, Claes; and Hellman, Bo (Histological Dept., Univ. of Upsala, Sweden): THE INFLUENCE OF THE LITTER SIZE ON THE POSTNATAL GROWTH OF THE ISLETS OF LANGERHANS IN THE RAT. Acta endocrinol. *35*: 535-40, December 1960.

The islet volumes of the pancreases of three-week-old rats were studied and found to be three times as large in the (larger) animals of small litters. This, however, was proportional to the size of the body and pancreas. The smaller absolute islet volume of the animals of the large litters showed a disproportionate reduction in the A cells. S.B.B.

Root, Howard F.; and Veit, Odette (Joslin Clinic, Boston, Mass.): DIABETIC EMERGENCIES. Journal-Lancet *80*:497-507, November 1960.

The mild, well treated diabetic individual when confronted by a medical emergency may present no serious problem aside from those inherent in the emergency itself. The unrecognized or poorly treated diabetic patient may add serious metabolic problems to those of the present emergency.

Diabetic nephropathy, if severe, may be responsible for edema, albuminuria, visual disturbance, hypertension, and albuminuria as well as retention of drugs administered in the treatment of an acute emergency.

Insulin hypoglycemia, its symptoms, etiology, and treatment are described along with a detailed account of corresponding aspects of diabetic coma. H.L.W.

Rosenkranz, A. (Dept. of Pediatrics, Univ. of Vienna, Austria): FRUCTOSE-METABOLISM IN ESSENTIAL FRUCTOSURIA. Klin. Wchnschr. *38*:1090-93, Nov. 1, 1960.

Essential fructosuria is a rare and usually harmless "inborn error of metabolism." It occurs about once in 120,000 births. It differs characteristically from the hereditary fructose intolerance (Froesch, Prader and Labhart). In essential fructosuria, fructose is tolerated well and there is no evidence of parenchymatous liver damage. An intravenous fructose tolerance test shows a delayed return of the fructosemia to the initial level without any changes in the serum inorganic phosphate level. In hereditary fructose intolerance, the fructose tolerance test is also delayed, but the serum inorganic phosphate is markedly decreased. In normal individuals there is a rapid disappearance of fructose from the blood after intravenous fructose loading and a moderate and short-lasting fall in the serum inorganic phosphate. In all three instances, the test is characterized further by a significant fall of the level of the glycemia. This, however, has only little differential diagnostic significance although in hereditary fructose intolerance the hypoglycemia may be most marked and prolonged (German). M.G.G.

Rothfeld, Edwin L.; Goldman, Jerrold; Goldberg, Harold H.; and Einhorn, Samuel (Newark Beth Israel Hosp., Newark, N. J.): SEVERE CHLORPROPAMIDE TOXICITY. J.A.M.A. *172*: 54-56, Jan. 2, 1960.

This case is of interest because of the severity and multiplicity of toxic effects of chlorpropamide and as the first report of a toxic ulcerative proctocolitis attributable to the drug. A fifty-three-year-old woman was treated with chlorpropamide after the initial diagnosis of diabetes mellitus. She took 500 mg. daily for five consecutive days and then 250 mg. daily. A pruritic skin rash and fever developed and within a few days she was hospitalized. Clinical and laboratory investigation disclosed an obstructive type of jaundice, erythema multiforme and many small ulcerations of the mucosa of the rectum and sigmoid compatible with a toxic ulcerative proctocolitis. Two days after admission the patient had a severe rectal hemorrhage which was treated with replacement of 1,000 mg. of whole blood. Because of the rapidly progressive downhill course she was placed on therapy with prednisone. Her skin lesion progressed to a generalized exfoliative dermatitis. After five weeks the steroid therapy was discontinued because of a psychosis which subsided in a week. The patient has remained well, and the diabetes is controlled with 20 units of NPH insulin daily. B.F.K.

Sokal, Joseph E. (Roswell Pk. Memorial Inst., Buffalo, N.Y.): THE DURATION OF GLUCAGON EFFECT. Endocrinology 67: 774-83, December 1960.

Glucagon inhibits liver glycogen deposition in the fasted animal given a glucose feeding. The duration of glucagon action was determined by measuring the interval between glucagon injection and the beginning of liver glycogen deposition in the rat tube-fed with glucose. Inhibition of glycogenesis was more prolonged with larger doses of glucagon and with zinc glucagon, and varied with the site of injection. Some effects of injected glucagon, such as that on the liver, may outlast other effects such as hyperglycemia. H.L.W.

Tomhave, Wesley G.; and Kuhl, W. James, Jr. (VA Research Hosp. and Northwestern Univ. Med. Sch., Chicago, Ill.): ORAL HYPOGLYCEMIC AGENTS. Arch. Int. Med. *106*: 345-53, September 1960.

The history, pharmacology, mode of action, clinical use and the toxicity of oral hypoglycemic agents are discussed. Tolbutamide excreted in the urine may cause a false positive test for urine protein when standard tests are employed. The only advantage of the drugs is one of convenience. B.F.K.

Volk, Bruno W.; and Lazarus, Sydney S. (Jewish Chronic Disease Hosp., Brooklyn, & Albert Einstein Coll. of Med., Bronx, N.Y.): RABBIT PANCREAS IN PROTEIN MALNUTRITION (EXPERIMENTAL KWASHIORKOR) AND AFTER CORTISONE ADMINISTRATION. Am. J. Path. 37:121-35, August 1960.

Protein deficient diets caused a fall in weight, loss of hair, pancreatic acinar atrophy and fatty metamorphosis with eventual cirrhosis of the liver. The hepatic lesions in some cases preceded the pancreatic changes and were attributed to the lack of protein in the diet rather than pancreatic dysfunction.

Cortisone administration resulted in pancreatic ductular proliferation and dilatation with inspissated luminal material, degranulation and glycogen infiltration of beta cells, and acinar atrophy.

The absence of ductular proliferation in the protein deficient animals suggested that this change seen in steroid treated animals was not due to the protein catabolic action of the hormone.

Peripancreatic fat necrosis and focal pancreatitis of obscure pathogenesis were seen in both dietary deficient and steroid treated animals. H.L.W.

Weinberg, Arnold N.; and Segal, Stanton (National Inst. of Arthritis & Metabolic Diseases, Nat'l. Inst. of Health, Bethesda, Md.): EFFECT OF GALACTOSE-1-PHOSPHATE ON GLUCOSE OXIDATION BY NORMAL AND GALACTOSEMIC LEUKOCYTES. Science *132*:1015-16, Oct. 14, 1960.

The author reports that during incubation with galactose, galactosemic leukocytes accumulated more galactose-1-phosphate than did normal leukocytes. Concomitant determination of glucose oxidation, with C^{14} glucose, revealed no inhibition of the hexosemonophosphate pathway. These results are at variance with recent studies in rat lens tissue, which suggest that intracellular galactose-1-phosphate depressed glucose-6-phosphate dehydrogenase activity and the oxidative pathway. The author points out that from the data obtained it cannot be concluded that glucose metabolism in other tissues is unaltered by the presence of Gal-1-P but the accumulation of this sugar is not necessarily associated with inhibition of the oxidative pathway of glucose metabolism. L.S.S.

Weinges, K. F.; and Schwarz, K. (II Dept. of Med., Univ. of Munich, Germany): THE INFLUENCE OF GLUCAGON, INSULIN AND GLUCOSE ON THE FREE PLASMA-17-HYDROXYCORTICOIDS OF MAN. Klin. Wchnschr. *38*:792-96, Aug. 15, 1960.

The fluctuations of the free 17-hydroxycorticoids were estimated in the plasma of normal subjects who had received either an intravenous injection of 0.02 mg./kg. glucagon, or an I.V. injection of 0.1 or 0.2 U./kg. Regular Insulin or an I.V. infusion of glucose over a period of thirty minutes with a concentration of 6-12 mg./kg./min. The method of Eik-Nes, Nelson and Samuels was used. Blood samples were withdrawn at fasting and 15, 30, 45, 60, 90, 120, 180 and 240 minutes after the injection of glucagon and insulin and after the termination of the glucose infusion. Each blood sample was also analyzed for its glucose content. Glucagon hyperglycemia was accompanied in each of the ten subjects by a significant transitory rise of the free 17-hydroxycorticoids, which reached a maximum of 73 per cent at the thirty-minute interval. After three to four hours the values returned to the fasting level. Likewise a transitory rise of the free plasma 17-hydroxycorticoids occurred simultaneously with insulin-induced hypoglycemia in eight individuals. Here a maximum of about +60 per cent was reached at the sixty-minute interval and the elevation persisted beyond the three-hour interval. In contrast, no change in the plasma level of the free 17-hydroxycorticoids was found after the glucose infusion, despite the fact that it resulted in a transitory hyperglycemia similar to that caused by glucagon.

Thus the glucagon induced increase of the plasma level of the free 17-hydroxycorticoids cannot be ascribed to the hyperglycemia. The authors believe it unlikely that this rise after glucagon is due to a stimulation of the adrenal cortex, which is supposed to be its cause in insulin hypoglycemia. They discuss the possibility that glucagon may affect the steroid metabolism in the liver and cite the case observation that in an instance of hepatic cirrhosis glucagon caused neither a hyperglycemia, nor an increase in the plasma level of the free 17-hydroxycorticoids (German). M.G.G.

Wilkerson, Hugh L. C.; Hyman, Harris; Kaufman, Mildred; McCuiston, Alvin C.; and Francis, John O.S. (Diabetes Field Res. Unit, Dept. of Health, Education, and Welfare, Brighton, Mass.): DIAGNOSTIC EVALUATION OF ORAL GLUCOSE TOLERANCE TESTS IN NONDIABETIC SUBJECTS AFTER VARIOUS LEVELS OF CARBOHYDRATE INTAKE. New England J. Med. *262*:1047-53, May 26, 1960.

Forty-two men and fifteen women, nondiabetic and apparently healthy, were studied to determine the effect of various amounts of carbohydrate on glucose tolerance, particularly in terms of diagnostic evaluation. The diets given, under supervision, varied from 300 to 50 gm. of carbohydrate. The 50 gm. diet was given to the men for three days and to the women for seven days. In no subject did a glucose tolerance test give a false positive diagnosis of diabetes. After seven days on a 50 gm. carbohydrate diet the women were given an isocaloric diet of either 100 or 200 gm. of carbohydrate for three days. On both diets the ketonuria that had been manifested on the previous diet was markedly decreased, and the slight decrease in glucose tolerance as judged by the one-hour sample tended to improve. It is suggested that the need for the routine use of preliminary high-carbohydrate preparation for a glucose tolerance test has been exaggerated. W.R.K.

Williams, Capt. Ralph C., Jr.; and Mahan, Major Charles J. (Massachusetts Gen. Hosp., Boston, Mass., and U.S.A.F. Hosp., Maxwell Air Force Base, Air Univ., Montgomery, Ala.): PERIODONTAL DISEASE AND DIABETES IN YOUNG ADULTS. J.A.M.A. *172*:776-78, Feb. 20, 1960.

Nine diabetic patients with extensive periodontal disease were selected for treatment. After stabilization of the diabetic program appropriate periodontal treatment and surgical procedures were carried out under local anesthesia as one procedure. No limitation was placed on the amount of tissue treated at one time. Penicillin and streptomycin were given for ten days. Seven of the nine patients showed significant reduction of the insulin requirement. It is suggested that careful oral evaluation and periodontal survey be conducted in every diabetic patient. B.F.K.

Williams, Wallace R.; Hill, R.; and Chaikoff, I. L. (Dept. of Physiol., University of California, Berkeley, Calif.): PORTAL VENOUS INJECTION OF INSULIN IN THE DIABETIC RAT: TIME OF INDUCTION OF CHANGES IN HEPATIC LIPOGENESIS, CHOLESTEROGENESIS, AND GLYCOGENESIS. J. Lipid Res. *1*:236-40, April 1960.

The time of onset of changes in liver metabolism of the fourteen-hour-insulin-deprived, alloxan-diabetic rat following continuous portal vein infusion of insulin was studied. The earliest effects of insulin—on glycogen content of the liver and on the capacity of the liver slice to synthesize cholesterol from acetate—occurred between ten and thirty minutes after the start of the infusion. The liver's capacity for incorporating acetate carbon into cholesterol rose dramatically between thirty and sixty minutes after the start of insulin-infusion, and returned to normal in ninety minutes. The significance of this biphasic response in hepatic cholesterogenesis to portal vein insulin infusion is discussed. Fatty acid synthesis was slowest to respond to insulin. An effect on hepatic lipogenesis was first detected between sixty and ninety minutes after injection, and the liver's capacity for incorporation of acetate-1-C^{14} into fatty acids continued to increase with duration of insulin administration up to 180 minutes. W.R.K.

Wills, M. R.; and Reece, M. W. (United Bristol Hospitals, Bristol, England): NON-CLOSTRIDIAL GAS INFECTIONS IN DIABETES MELLITUS. Brit. M. J. *2*:566-68, Aug. 20, 1960.

Four fatal cases of nonclostridial gas infections in diabetics are described and the literature is briefly reviewed. J.A.

Wilson, Reginald A.; and Clark, Nigel (Dept. of Paediatrics, Vancouver Gen. Hosp., and Faculty of Medicine, Univ. of British Columbia, Vancouver 9, B.C.): ENDOCARDIAL FIBRO-ELASTOSIS ASSOCIATED WITH GENERALIZED GLYCOGENOSIS. Pediatrics *26*:86-96, July 1960.

Case histories of three siblings who died with glycogen storage are presented. The two who survived longest were found at autopsy to have endocardial fibro-elastosis and this was more advanced in the oldest infant. The infant who survived the shortest period of time had no evidence of fibro-elastosis. It is suggested that fibro-elastosis develops after long exposure of the myocardium to high levels of glycogen. The biochemistry of the group of disorders included under the term glycogen storage disease is discussed. R.L.J.

Windom, Robert E.; Bass, Robert K.; McBride, Robert B.; and Sanford, Jay P. (Dept. of Internal Medicine, The Univ. of Texas Southwestern Med. School, Dallas, Tex.): ROUTINE URINE CULTURES IN PRIVATE PRACTICE. Am. J. M. Sc. *241*: 14-17, January 1961.

The technic of sterile-voided urine collections combined with a quantitative culture technic has been practical for the collection of specimens from females for bacteriological study in a medical practice. Significant bacteriuria (50,000 to 100,000 bacteria/cc. of urine) was detected in only one of 100 unselected, ambulatory, nonindigent female patients. Thirteen of these patients had a past history of urinary tract disease and seven had sustained diastolic hypertension. This frequency is lower than observed in most other studies in which either hospitalized or medically indigent patients were studied. L.S.S.

Yalow, Rosalyn S.; and Berson, Solomon R. (Radioisotope Service, Veterans Administration Hosp., New York, N.Y.): IMMUNO-ASSAY OF ENDOGENOUS PLASMA INSULIN IN MAN. J. Clin. Invest. *39*:1157-75, July 1960.

A precise method of immuno-assay of endogenous plasma insulin is described. It is based on the use of guinea pig anti-beef insulin antibody to detect human insulin competing with beef-insulin-I^{131}. Paper chromatoelectrophoresis is used to separate antibody-bound insulin from unbound insulin. This method is sensitive to less than one micro-unit of insulin. The plasma from normal humans and maturity onset diabetic patients were compared as to insulin content both during fasting and the course of a standard two-hour glucose tolerance test. The mean fasting level was higher in the diabetic (27 micro-units) than in the normals. The average insulin concentration during the two-hour test was likewise higher in the plasma of the diabetic patients (147) as compared with the normals (117). A large proportion of diabetic patients reached the peak of plasma insulin concentration later than the normals (one-half to one hour).

Other patients studied were those with cirrhosis, acromegaly and thyrotoxicosis. Patients with islet cell adenoma had high elevated fasting plasma insulin levels, but responded normally to a glucose tolerance test. Of six patients who developed hypoglycemia after leucine four showed an increase in plasma insulin.

From the studies, the average secretion of insulin per day was estimated as 55 units in the normal adult. S.B.B.

Zollinger, Hans U. (Dept. of Path., Kantonsspital St. Gallen, Switzerland): NECROSIS OF THE RENAL PAPILLAE IN DIABETES. German M. Month. *5*:332-34, October 1960.

Necrosis of the renal papillae is not pathognomonic of diabetes; it also occurs in pyelonephritis and chronic interstitial nephritis. However, the combination of diabetes and pyelonephritis often causes papillary necrosis. This is due not only to the increased liability of diabetics to infections, but also to vascular damage. Suggestions towards clinical diagnosis are made, in particular the histological examination of sloughed papillae passed in the urine. Recovery may take place, the stumps of the papillae being covered by new epithelium. W.R.K.

ORGANIZATION SECTION

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LAURANCE W. KINSELL, M.D., *Oakland*
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TERM EXPIRING 1962

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MAURICE PROTAS, M.D., *Washington, D.C.*, Chairman, Assembly of Delegates

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21st ANNUAL MEETING JUNE 24-25, 1961

The Twenty-first Annual Meeting of the American Diabetes Association will be held at the Hotel Commodore in New York City, June 24-25, 1961, immediately prior to the annual session of the American Medical Association. The Scientific Sessions will be presented on these two dates, which fall on Saturday and Sunday. Jerome W. Conn, M.D., is Chairman of the Committee on Scientific Programs, whose members include Blair Holcomb, M.D., Vice Chairman; James Ashmore, Ph.D.; Thaddeus S. Danowski, M.D.; Irving Graef, M.D.; and Harvey C. Knowles, Jr., M.D.

Among the highlights of the Sessions:

- *Rachmiel Levine, M.D.*, will deliver the Banting Memorial Lecture. His subject will primarily concern insulin action. Dr. Levine is Chairman and Professor, Department of Medicine, New York Medical College, Director, Medical Services, Flower and Metropolitan Hospitals, New York.
- *Rosalyn S. Yalow, Ph.D.*, will receive the 1961 Lilly Award. Dr. Yalow, who is Assistant Chief, Radioisotope Service, Veterans Administration Hospital, Bronx, New York, has been instrumental in developing a sensitive immunoassay technic for determination of insulin in biological materials. In addition, she will present a paper on "Immunoassay of Insulin" at the Annual Meeting.
- *Dr. Ralph Robey* will be the principal Banquet speaker. Formerly Financial Editor of *Newsweek*, Dr. Robey is Economic Research Director of the National Association of Manufacturers.

Members and their wives (or husbands) and friends are invited to attend the Annual Banquet and Social Hour Saturday evening, June 24. A reservation form for the Banquet will be mailed during the month of May.

Pertinent information about the Annual Meeting, together with a hotel reservation form, was mailed to members of the Association in November. Those who have not yet made hotel reservations are urged to do so immediately by filling out the form and sending it directly to the Hotel Commodore.

Members who plan to attend the ADA Annual Meeting and wish to stay over at the same hotel for the annual meeting of the American Medical Association on June 26-30, must inform the Hotel Commodore that they would like to continue their reservation for the AMA sessions.

DIABETES will publish a list of papers to be given at the Scientific Sessions; and the program itself, including abstracts of all papers presented or read by title, will be sent to all members before the meeting.

There is no registration fee for members of the Association. Fee for nonmembers is $2.00, which includes a copy of the program.

Members and nonmembers may secure additional copies of

the program at a cost of $1.00 each by requesting them from the American Diabetes Association, 1 East 45th Street, New York 17, N. Y. The program will also be available at the Registration Desk in New York for those who want to purchase one at the time of the Meeting.

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June 9-10, 1962, is the date of the Twenty-second Annual Meeting. It will be held in Chicago at the Conrad Hilton Hotel.

10th POSTGRADUATE COURSE

The American Diabetes Association will present its Tenth Postgraduate Course Jan. 17, 18 and 19, 1962, in Detroit, Michigan. Further information concerning the Course will be published in forthcoming issues of the Journal.

1962 LILLY AWARD

The sixth Lilly Award will be given at the Twenty-second Annual Meeting of the American Diabetes Association June 9-10, 1962, in Chicago. The following stipulations govern the contest for this annual award, which is supported by Eli Lilly and Company and consists of $1,000 and a medal.

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before December 1 of the year preceding the award. The nomination should be accompanied by full information concerning the nominee's personality, training and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to December 1 of the year preceding the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

AMA DIRECTORY TO LIST ADA MEMBERSHIP

The American Medical Association has approved inclusion of the American Diabetes Association in the group of "Special Societies" used in the listing of individual physicians and their membership in professional societies in the Twenty-first (1960-61) edition of the *American Medical Directory*. It is anticipated that this edition will be available in the spring of this year.

AMA JOURNAL TO PUBLISH *DIABETES* ABSTRACTS

The Journal of the American Medical Association will publish abstracts of articles appearing in DIABETES: *The Journal of the American Diabetes Association,* beginning in an early issue. The selection of DIABETES as one of the "leading journals of the United States to participate in the program of abstracts" is part of the American Medical Association's plan to develop an extended and more effective abstract coverage. The goal will be to publish abstracts within one month following publication of the original articles; and periodically to print an index with reference to the parent journal, subject and authorship.

4th CONGRESS OF THE INTERNATIONAL DIABETES FEDERATION: July 10-14, 1961

As previously announced, the International Diabetes Federation will hold its Fourth Congress in Geneva, Switzerland, July 10-14, 1961. The preliminary program and other details were published in the January-February issue of DIABETES.

Those interested in group transportation by chartered plane should communicate as soon as possible with Howard F. Root, M.D., 15 Joslin Road, Boston 15, Mass. Round-trip fare is available at approximately $300. The plane will leave New York in the late morning of Saturday, July 8, 1961, will stop at Boston, and will return from Paris four weeks later.

NEW MEMBERSHIP DIRECTORY

The American Diabetes Association will publish an up-to-date Membership Directory with the names of all members of record as of March 1, 1961. The Directory will include an alphabetical and geographical listing of Active Members, an alphabetical listing of Associate and Corporate Members, and other information of organizational interest. It will be mailed to all members without charge.

Communications were mailed to all Active and Associate Members of the Association on March 1 and March 6 respectively, requesting information for inclusion in the Membership Directory. The communication was sent via First Class Mail, and by Air Mail overseas. Replies must be received no later than March 31, 1961.

SUMMER CAMP LIST IN ADA *FORECAST*

Physicians and other interested persons may wish to call the attention of diabetics and their families to the list of "Summer Camps for Diabetic Children" scheduled for the May-June

FORECAST. Sponsorship of the camp will be given as well as camping dates, rates, ages of children accepted at particular periods, and the name and address of the person to contact. Copies of the May-June issue may be obtained after April 15 at 35¢ each or six for $2.00. Single copies of reprints of the camp list may be secured without charge.

DIABETES WEEK 1961

Diabetes Week will be observed Nov. 12-18, 1961, following the usual practice of observing official Diabetes Week the week before Thanksgiving. Affiliate Associations and Committees on Diabetes of County and State Medical Societies are urged to note this date in making plans for their Public Education and Detection programs.

NEW MEMBERS

The following were elected as of Feb. 1, 1961:

Illinois	
Kelly, Frank B., Jr.	Chicago
Indiana	
Bromer, William W.	Indianapolis
Petitjean, H. G.	Haubstadt
Kentucky	
Harrison, M. M.	Louisville
Massachusetts	
Stellar, L. I.	Newton
Michigan	
Mandiberg, J. N.	Detroit
New Jersey	
Kass, Morton	Paterson
Riccioli, N. F.	Clifton
New York	
Chen, Wei	New York
Ohio	
Macht, Martin B.	Cincinnati
Pennsylvania	
Galloway, John A.	Philadelphia
Rhode Island	
McWilliams, J. G.	Providence
Wisconsin	
Meyer, J. M.	Milwaukee
Other Countries	
England	
Redhead, Ian H.	Northants
Walker, Geoffrey	London

NEWS OF AFFILIATE ASSOCIATIONS

THE BIRMINGHAM METABOLIC AND DIABETES ASSOCIATION will hold a meeting March 19, 1961, sponsored conjointly with The Alabama Diabetes Association and the Department of Medicine of the Medical College of Alabama. Alexander Marble, M.D., Boston; Harvey C. Knowles, Jr., M.D., Cincinnati; George M. Guest, M.D., Cincinnati; and James Ashmore, Ph.D., Indianapolis, will speak on the program.

The CHICAGO DIABETES ASSOCIATION held its annual meeting Feb. 3, 1961, at which the following directors were elected for terms expiring in February 1964: Donald I. Bell, M.D., Arthur R. Colwell, Sr., M.D., Ford K. Hick, M.D., William S. Hoffman, M.D., Mr. L. Duncan Lloyd, Mr. William A. Morse, Henry T. Ricketts, M.D., Mrs. Beverly Schmidt, Mrs. M. J. Spiegel, Charles S. Vil, M.D. At the board meeting following the annual meeting, these officers were elected for terms expiring in February 1962: M. David Allweiss, M.D., President; R. Lincoln Kesler, M.D., 1st Vice-President; Carl A. Hedberg, M.D., 2nd Vice-President; Ralph E. Dolkart, M.D., Secretary; Mr. George K. Dahlin, Treasurer.

The DELAWARE DIABETES ASSOCIATION held a meeting of the Lay and Clinical Societies February 16 in Wilmington at which Joseph T. Beardwood, Jr., M.D., Philadelphia, chairman of the Committee on Employment of the American Diabetes Association, discussed aspects of employment for diabetics. "Life Insurance for Diabetics" was also presented by Paul S. Entmacher, M.D., New York.

The DIABETES ASSOCIATION OF SOUTHERN CALIFORNIA held a meeting on February 2, at which Stefan S. Fajans, M.D., spoke on recent work at the University of Michigan Medical Center on the uses and mechanisms of action of sulfonylureas and leucine in various hypoglycemic states. Dr. Fajans, who is Associate Professor of Internal Medicine at the University of Michigan, also described clinical interpretations of intravenous tolbutamide tests.

The LOUISIANA DIABETES ASSOCIATION will display the ADA scientific exhibit entitled "Diabetes—Today and Tomorrow: The Expanding Role of the Doctor" at a meeting of the Louisiana State Medical Society in New Orleans May 8-10.

The PITTSBURGH DIABETES ASSOCIATION will hold a dinner meeting of the Lay and Clinical Societies on April 18, at which Franklin B. Peck, Sr., M.D., President of the American Diabetes Association, will speak on "Diabetes Abroad."

The VIRGINIA DIABETES ASSOCIATION will hold its annual meeting in Washington, D.C., on May 12, 1961, at the Sheraton Park Hotel. The theme of the program will be "How to Find Early Diabetics and How to Treat Them." Scheduled speakers are: Hugh L. C. Wilkerson, M.D., Boston, "Testing for Diabetes"; Max Miller, M.D., Cleveland, "Benefits That Accrue to Patients from Early Diagnosis of Diabetes"; William R. Jordan, M.D., Richmond, "Clinical Situations Commonly Associated with Diabetes." Drs. Wilkerson, Miller and Jordan will participate in a panel discussion moderated by Robert K. Maddock, M.D., Norfolk, President of the Virginia Diabetes Association.

CAREFUL PLANNING

In order to maintain a comprehensive, long-range national program, the American Diabetes Association must plan its work carefully. Basically, of course, the continuity and future effectiveness of the program depend on the interest and foresight of the Association's individual members.

Every member can express his interest by planning his estate to take maximum advantage of benefits resulting from charitable bequests. A bequest to the American Diabetes Association is exempt from estate taxes and may have many advantages for the member's family or heirs. And primarily, of course, it is an investment in the well-being of present and future generations of diabetics.

The following form of general bequest contains some basic information which may be helpful to your attorney:

"I hereby give and bequeath to the American Diabetes Association, Inc., a corporation organized under the laws of the State of Ohio, and having its principal office at 1 East 45th Street, New York 17, New York, the sum of . . . . . dollars."

Your attorney will also be able to discuss with you the methods for giving real property, as well as cash or securities.

If additional information is desired, it will be made available immediately by the American Diabetes Association, 1 East 45th Street, New York 17, New York.

NEWS NOTES

44th ANNUAL MEETING OF THE AMERICAN DIETETIC ASSOCIATION

Oct. 24-27, 1961, is the date of the 44th Annual Meeting of The American Dietetic Association in St. Louis. The Sheraton-Jefferson will be the headquarters hotel, and the Kiel Auditorium will be the location for sessions and the exhibition.

ADA MEMBERS PARTICIPATE IN ACP POSTGRADUATE COURSES

The following members of the American Diabetes Association will take part in postgraduate courses of The American College of Physicians this spring:

Postgraduate Course No. 8, "Internal Medicine: Selected Topics," at McGill University School of Medicine in Montreal March 13-17: JOHN C. BECK, M.D., "Aldosterone and Renal Function"; Chairman "Endocrine-Metabolic Section"; "Current Trends in the Management of Thyroid Disorders"; "Metabolic Actions of Human Growth Hormone in Man—Its Possible Clinical Applications"; "Management of Endocrine Dependent Carcinemia." ALLEN GOLD, M.D., "The Role of the Oral Hypoglycemic Agents in the Management of Diabetes." MARTIN M. HOFFMAN, M.D., "Management of Endocrine Dependent Carcinemia"; "Intestinal Malabsorption." GUY JORON, M.D., "Pyelonephritis: Pathogenesis and Predisposing Factors." LESLIE KOVACS, M.D., "The Role of the Oral Hypoglycemic Agents in the Management of Diabetes." ELEANOR E. MCGARRY, M.D., "The Application of Immunochemical Technics to the Measurement of Protein and Polypeptide Hormones"; "The Role of the Oral Agents in the Management of Diabetes." WALTER DEM. SCRIVER, M.D., Chairman of Renal Section.

Postgraduate Course No. 10, "Endocrinology and Metabolism," University of Virginia Medical Center in Charlottesville, March 23-25, 1961: H. ST. GEORGE TUCKER, M.D., Moderator of afternoon session March 23. H. B. MULHOLLAND, M.D., Moderator of Saturday morning session March 25. JOHN A. OWEN, JR., M.D., "Panel of Questions and Case Reports"; "Metabolic Bone Disease"; "Diabetes Mellitus—Current Concepts."

Postgraduate Course No. 11, "Problems of Growth and Aging," The Lankenau Hospital in Philadelphia April 11-14, 1961: EDWARD L. BORTZ, M.D., Director, and chairman of afternoon session April 14. HENRY F. PAGE, M.D., Associate Director, and Chairman of Wednesday morning session April 12. WALTER M. BORTZ, II, M.D., "Enzyme Factors in Lipid Metabolism."

PERSONALS

J. A. CAMPBELL, M.D., Ottawa, spoke on "Some Factors Affecting the Absorption of Vitamins" at the "Symposium on Mechanisms of Gastrointestinal Absorption" held March 7 in conjunction with the Sixteenth Annual Meeting of The National Vitamin Foundation.

HERBERT POLLACK, M.D., New York, will speak on "Malnutrition" May 10, 1961, as one of a series of lectures on clinical nutrition at the Philadelphia General Hospital. The lectures have been arranged by the Committee on Nutrition and Metabolism of the Philadelphia County Medical Society jointly with the National Vitamin Foundation and the Department of Education of the hospital.

HENRY T. RICKETTS, M.D., Chicago, has been named to the editorial board of the *Archives of Internal Medicine.* A Past President of the American Diabetes Association, Dr. Ricketts is a member of the Editorial Board of DIABETES.

ROSALYN S. YALOW, PH.D., New York, winner of the 1961 Lilly Award, was one of six women who received the first annual award of the Civil Service Commission February 24 for her pioneering in using radioisotopes in medical tracer work.

NECROLOGY

HELEN M. MCEVOY, Brooklyn, New York, born August 18, 1919.

ELDON S. MILLER, Kansas City, Kansas, born September 24, 1903.

ARTHUR L. WALTERS, Miami Beach, Florida, born January 20, 1884.